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SOIL TEMPERATURE VERSUS DROUGHT AS A FACTOR DETERMINING LOWER ALTITUDINAL LIMITS OF TREES IN THE ROCKY MOUNTAINS

R. F. DAUBENMIRE

Introduction

Each of the various species of coniferous trees which dominate the Rocky Mountain forests has rather definite limits of altitude above and below which it normally does not grow, and these vary considerably among species. It seems safe to assume that the factors which govern the upper altitudinal limits of a tree are in all probability different from those which effect the lower limits of its range. For example, if the summer temperature above a certain elevation is insufficient to meet the heat requirements of a particular species, this factor cannot be effective in the same way at the lower limit of the species range, since temperature increases down the slope. It is therefore feasible to ignore one set of factors, such as those involved in determining the upper altitudinal limits, and concentrate attention on the other set. This paper is concerned primarily with the two factors most frequently suggested as playing a critical role in determining the downward limits of distribution, namely, high temperature and drought.

The earliest important contributor to the geography of the montane vegetation of the Rocky Mountain region was C. H. MERRIAM, who considered that the lower altitudinal limits of plants are determined by their varying abilities to endure midsummer heat. This critical degree of heat he contended could be expressed as the mean temperature of the six hottest consecutive weeks in summer. His assumption was wholly unsupported by experimental or circumstantial evidence, and although it was long accepted without question, the hypothesis (7) is now generally discredited.

Later investigators made sufficient measurements of the environmental factors associated with the zonal sequence of vegetation to hazard a guess as to which factor is most likely limiting in effect. Also, the interpretation of species behavior at the lower limits of altitudinal distribution proved an additional source of information. It is significant that practically all these contributors (5, 10, 12, 16, 22, 23, 25, 26, 27, etc.) concluded that drought is more important than high temperature in determining lower altitudinal limits. BATES (1), however, considered lack of shade for the seedlings the factor most limiting in the reproduction of Douglas fir (*Pseudotsuga taxifolia*) at its lower altitudinal limits.

Experimentation has been employed in connection with Rocky Mountain vegetation zones only within the last two decades. To date two investigators, BATES and PEARSON, have made the principal experimental contributions to the etiology of this zonal system, but their results have not led to the same conclusions with respect to lower altitudinal limits. PEARSON held that the lower limits of distribution are determined by the varying abilities of species to withstand drought, while BATES placed more importance on high temper-

ature at the soil surface. The present experimental study attempts to determine the relative importance of these two factors.

By exposing flats of seedlings to strong insolation, BATES (2) observed differences in mortality which indicated that seedlings of Engelmann spruce (*Picea engelmannii*) and lodgepole pine (*Pinus murrayana*) are more susceptible to high soil surface temperatures than are Douglas fir and ponderosa pine (*Pinus ponderosa*). This finding seems to have significance in connection with distribution, in view of the fact that the latter two species range down to distinctly lower elevations in the Rockies than do the former.

Later, by using a different technique, somewhat different results were obtained (4). In these experiments potted seedlings were exposed successively when they were 46, 64, 90, and 92 days old to heat radiated from a resistance coil mounted under a reflector. To make sure that soil drying was not a cause for mortality, the soil in the pots was watered so thoroughly that its surface temperature never became as hot during the subsequent tests as did the adjacent layer of air, and in all cases foliage injury rather than stem injury at the ground level was observed. This second study, therefore, resolved itself to tests of the relative abilities of different species of seedlings to tolerate the effects of hot air about their foliage. According to the results obtained, the species could be rated as to sensitiveness as follows (the figures which follow the names indicate the order in which the species are normally encountered in ascending a slope):

Most sensitive:	Lodgepole pine.......	3
	Ponderosa pine.......	1
	Englemann spruce....	4
Least sensitive:	Douglas fir..........	2

Since in this experiment the relative tolerance of the foliage of the seedlings to high temperature bears no relation to their zonal distribution, it would appear that this aspect of high temperature is not critical in determining the lower altitudinal limits of the species. However, 2 years later BATES (3) still maintained that the forest zones of the central Rockies "are essentially temperature belts and only secondarily moisture belts," and explained the difference in the lower altitudinal limits of Douglas fir and ponderosa pine as due to an ability of seedlings of the latter species to tolerate higher levels of temperature at the soil surface. The temperature at this level, he stated, is "far more important" than air temperatures.

A contrasted view is held by PEARSON (19). He transplanted small trees of Douglas fir and blue spruce (*Picea pungens*), which have approximately the same altitudinal distribution, into the ponderosa pine zone. All transplants succumbed in such a manner as to lead PEARSON to conclude that drought was the causal factor, and he explains the differing drought tolerance among species as due to the relative depth of the root systems in relation to the periodic desiccation of the upper soil horizons. Moreover, he pointed out that all forest trees native to the southern Rockies can grow at altitudes much lower than they naturally occur if they are watered adequately.

It was the purpose of the research reported in the present paper to make comparative studies under controlled conditions of both heat and drought tolerance of certain of the more important coniferous tree species of the Rockies, and then, where advisable, to compare these results with measurements of the same factors under field conditions. The former

type of study should provide a quantitative physiological basis for evaluating the relative importance of the accumulated field data and thus contribute information which may help to clear up the existing uncertainty concerning this phase of forest distribution.

Investigation

Relative tolerance of high soil-surface temperatures

Methods.—The species used in this study, together with the seed source, in the order of their zonal relationships, are as follows:

Subalpine fir (*Abies lasiocarpa*)—San Isabel National Forest, Colorado
Engelmann spruce—San Isabel National Forest, Colorado
Douglas fir—McCall, Idaho[1]
Ponderosa pine—McCall, Idaho
Piñon (*Pinus edulis*)—Colorado

The seeds were planted in a heavy sandy loam surfaced with 1 cm. of sand. At the time of planting, cupric oxalate was applied to the soil surface at the rate of 5 gm. per square foot to minimize losses due to damping-off fungi, and the soil was soaked with a complete nutrient solution at that time.

The flat measured 20 × 120 cm., and the seeds were sown in mixed rows approximately 2 cm. apart down the length of the flat. Just before the tests were begun all misformed and under-developed seedlings were removed, and the remaining plants were thinned so that no part of the soil surface would be especially shaded by the foliage of a clump of seedlings.

The duration of the heat treatments was arbitrary, although an attempt was made to simulate field conditions. Seedlings which germinate in forest openings are subjected each day to periods of insolation, the lengths of which depend upon the size and position of nearby objects which can intercept direct sunlight. During the day the temperature of the soil surface rises and falls with each moving shadow and passing cloud. To approximate these conditions in the greenhouse, each temperature test was conducted for a 6-hour period in the middle of the day, and at an average of once every 5 minutes the heating apparatus was connected until the surface temperatures rose to the desired level, at which time it was disconnected to prevent overheating. Thermostatic control of heat may have had some advantages in this connection, but sustained high temperature levels would be very unlike field conditions; moreover, thermoregulators to control true surface temperatures were unavailable. The temperature—even a few millimeters above or below the soil surface—is very different from that of the surface layer of soil particles, and it is the temperature of this surface which is critical. Temperatures were measured with a copper-constantan thermocouple,[2] with the measuring junction barely covered by the surface layer of sand grains. The accuracy of this instrument was checked by use of hot-water bath immediately before each test. Throughout each 6-hour period the writer personally tended the apparatus, bringing the temperature up to the desired level every few minutes.

Heat treatments were begun when the seedlings were 47 days old. The tests were made on alternate days, each time elevating the temperature level by 5° C.,

[1] Acknowledgment is hereby made of the generous co-operation of Mr. Royale K. Pierson, Mr. T. B. Glazebrook, and Mr. J. E. King of the School of Forestry, University of Idaho, for supplying and stratifying certain of the seeds used in this study.

[2] The writer is greatly indebted to Dr. Hobart Beresford and Mr. N. B. Akesson, for the loan of the heat-producing and measuring equipment.

until most of the plants had succumbed. The flat was watered only at the conclusion of each test, but at this time sufficient water was applied so that the subsoil remained too moist for drought to become a factor at any time, yet the surface layer of sand was dry enough on the second day after watering so that evaporation did not prevent the attainment of the desired temperature level. The 2-day interval gave ample time for the appearance of symptoms showing that a seedling had been fatally injured by a particular heat level.

With this method of conducting temperature tolerance tests, the first treatments probably influenced the effectivity of the subsequent ones by increasing the seedlings' heat resistance. This being the case, the procedure followed would seem very necessary if the results are to be used in connection with field problems, for it parallels the conditions to which seedlings are exposed in nature. Most seeds germinate in April and May, when the soil is moist, so that day by day as the soil dries out they are subjected to increasingly higher levels of soil-surface temperature.

The long flat was divided in half crosswise, and in each end a different method of soil heating was employed. Over one end was suspended a battery of four heating lamps which give off a high proportion of infra-red wave-lengths. For the other end of the flat a lead soil-heating cable, 6.5 mm. in diameter, was shaped into a grid, the segments of which extended back and forth between the rows of seedlings. This cable was put into position before the seeds were planted to avoid disturbance of the seedlings and was pressed down until about half the cable was below the soil surface. During the tests a thick wooden board was suspended 1 dm. above the lead cable, so that the seedlings in this end of the flat were screened from the heating lamps suspended over the other end.

Although different methods of soil heating were used during the tests, which were run simultaneously, the same temperature levels were maintained in both ends of the flat. The purpose of using two methods was to ascertain whether high surface temperatures per se, as maintained with the cable, are equally as important as high surface temperatures in conjunction with heating of the foliage as was the case under the lamps.

Despite all attempts to maintain uniformity of temperature over the flat, a certain amount of variation could be detected by moving the measuring junction of the thermocouple over the surface. Under the lamps the shade cast by the foliage of each seedling lowered the temperature a few degrees about the immediate base of the seedling. This problem was in part solved by thinning the stand of seedlings, as mentioned previously, and in part by using four heating lamps arranged in a row, so that the soil at the base of each seedling received heat rays from at least three angles. It is highly probable that some of the irregularities noted in seedling mortality may have been due to this factor, although the individual variations in tolerance are indeed great. Nevertheless, the differences among species proved to be so great, and the results obtained by the different methods of applying heat were so similar, that the experimental error appears to be negligible.

RESULTS.—The temperature tolerance tests (table 1) show marked differences among most of the species in their ability to tolerate high soil-surface temperatures, and these differences are correlated to a certain extent with the relative altitudinal distribution of the species: the

greater the tolerance of heat, the lower the natural occurrence of the tree. Fir and spruce, whose altitudinal ranges are so similar that they are generally considered dominants of the same (subalpine) forest belt, began to show injury between 40° and 45° C. Douglas fir and ponderosa pine resisted injury until temperatures of 55°–60° were attained, while piñon remained uninjured at temperatures of 70° C. Even in species relatively sensitive to heat a few seedlings were able to develop extraordinary resistance. For example, 17 per cent of the subalpine fir seedlings withstood the highest temperatures employed.

Nearly identical results were obtained with the two methods of heating, a fact which strongly suggests that the injurious effects of insolation are truly the result of heat injury at the base of the stem rather than the result of excessive absorption of heat by the foliage. Further evidence substantiating this conclusion is that with both methods of heating, symptoms of heat injury (a grayish discoloration of the lower 2 mm. of the stem) appeared simultaneously.

The temperature levels which proved lethal in this experiment vary considerably from values obtained by other investigators, and the latter are by no means in accord. Heretofore it has generally been thought that most coniferous trees of temperate North America have approximately the same degree of tolerance of soil-surface temperatures, and various investigators have reported injurious temperatures as beginning at 50° C. (24), 52° C. (14), 60° C. (14), and 62° C. (13). In the writer's experiments injury began at 40°–45° for certain species, while one species apparently remained uninjured at 70° C. Using seedlings 39–58 days old, ROESER (21) found injury to begin in Engelmann spruce at 51° C., in Douglas fir at 53°, and in ponderosa pine at 50°. According to his results there is little difference among the species as to their temperature tolerance, and this difference is not related to altitudinal range. Possibly some of the di-

TABLE 1

COMPARISON OF SURVIVAL OF SEEDLINGS DURING EXPOSURES TO INCREASINGLY HIGHER SOIL-SURFACE TEMPERATURE (° C.) LEVELS FOR 6-HOUR PERIODS

Heating equipment	Species	No. of seedlings	Cumulative mortality at different temperature levels (%)						
			40°	45°	50°	55°	60°	65°	70°
Heating lamps	Picea engelmannii	59	0	3	19	46	58	82	94
	Abies lasiocarpa	17	0	6	18	24	36	83	83
	Pseudotsuga taxifolia	17	0	0	0	6	6	24	36
	Pinus ponderosa	20	0	(10)*	(5)	0	(15)	25	30
	Pinus edulis	21	0	0	0	0	0	0	0
Soil cable	Picea engelmannii	62	0	11	48	82	82	†	......
	Abies lasiocarpa	11	0	9	9	36	36	......	......
	Pseudotsuga taxifolia	15	0	0	0	7	27	......	......
	Pinus ponderosa	9	0	0	0	11	11	......	......
	Pinus edulis	21	0	0	0	0	0	......	......

* Mortality due to damping-off rather than to heat treatment and therefore not included with cumulative mortality due to heat. Of all the species studied this pine has consistently proved the most susceptible to damping-off under greenhouse conditions.

† The soil cable proved inadequate for obtaining soil-surface temperatures above 60° C.

versity between his results and the writer's may be attributed to differences in technique; with ROESER's technique the seedlings were heated in a manner which differed far more than the writer's from natural conditions.

It is also likely that much of the discrepancy in results with these tests is due to differences in methods of measuring surface temperatures. Soil "surface" temperatures have been variously measured—by laying the sensitive element of the thermometer (commonly 5–27 mm. in diameter) on the surface, by partly imbedding it, or by burying it just deep enough so that its surface is thinly covered with soil. Such methods lead to widely different conclusions, for under strong insolation there is an extremely sharp temperature gradient in both directions from the soil surface. Using a thermocouple, the writer has found that under strong insolation the air 5 mm. above and the soil 5 mm. below the soil surface may be 15.0° and 5.3° C. cooler, respectively, than the surface itself. In view of this condition it is difficult to see how a thermometer with a bulb 5 mm. in diameter could be used to measure true surface temperatures accurately. Certainly no temperature is as significant as the maximum, and that is obtained by the very surface layer of particles. The thermocouple is the only instrument adequate for measuring such temperatures, since the sensitive part, the juncture of two fine wires, is less than 1 mm. in diameter. It may be concluded that possibly many of the data quoted as critical soil-surface temperatures for seedling survival are hardly more than crude approximations.

Among the species employed in this series of studies, those with thin-stemmed seedlings grow at high elevations, while the pines, especially piñon, which have relatively thick stems, grow at low elevations. It might appear that differences in tolerance of high soil-surface temperatures, and hence lower altitudinal limits, could be explained on the basis of relative thickness of insulating tissues in the vicinity of the soil surface. However, close analysis of the survival data in relation to stem anatomy, together with evidence provided by the interpretation of species distribution, militate strongly against this hypothesis.

In spruce, subalpine fir, and Douglas fir all bark tissues were still living at the time the experiments were performed. Cross sections of the stems just above the soil surface showed that subalpine fir and Douglas fir had approximately equal stem diameters and bark thicknesses, yet these species showed distinctly different abilities to tolerate hot soil surfaces. On the other hand, subalpine fir seedlings exceeded those of spruce in stem thickness and bark thickness, but both species exhibited the same degree of heat resistance. Further evidence that stem morphology of seedlings is not very well correlated with altitudinal distribution is provided by the fact that the thick-stemmed white-bark pine (*Pinus albicaulis*) is limited to high elevations, while the thin-stemmed junipers are best represented near lower timberline.

EVALUATION OF RESULTS.—It was not feasible in the field to ascertain directly whether or not the maximal soil-surface temperatures just below the natural limits of distribution of each species are everywhere in excess of its tolerance, as found in the greenhouse tests. A priori, such tests seem unnecessary for three reasons.

First, it is well known that even a thin shade will keep the temperature of the soil surface lower than that of the air, for the air is the source of practically all the

heat which a shaded surface may come to possess. KORSTIAN and FETHEROLF (15) found that high soil-surface temperatures caused lesions at the ground line in seedlings of Engelmann spruce when planted below their natural elevational limits in Utah, but they also observed that even a slight shading of the soil at the base of the stem, caused by an inclination of the seedling's axis toward the south, greatly reduced mortality from this cause. As PEARSON (19) pointed out, if maximal temperatures were the factor which prevents Engelmann spruce from advancing downslope into the territory occupied only by Douglas fir, one would expect to find spruce seedlings becoming established under the dense canopies of young Douglas fir stands, or at least in shaded nooks formed by the fortuitous juxtaposition of trunks, fallen logs, rocks, etc. The fact that there is an abundance of micro-habitats in each forest zone which are perpetually sheltered from direct sunlight seems to constitute excellent evidence that none of the species at their lower altitudinal limits have attained a point below which they cannot extend because of an intolerable degree of heat.

A second type of evidence which militates strongly against the temperature hypothesis is the fact that the School of Forestry of the University of Idaho maintains a nursery in which seedlings of *Pinus monophylla*, ponderosa pine, Douglas fir, and subalpine fir may be grown entirely without shade (20). The site of this nursery is on the basal plain below lower timberline, and the midsummer season here is characterized by strong insolation. The only special care which the seedlings require is frequent sprinkling, for the months of July and August have exceedingly low rainfall.

Lastly, the laboratory tests revealed no significant difference in the temperature tolerance of ponderosa pine and Douglas fir seedlings, yet throughout those parts of the Rockies where both species occur there is a distinct difference in their altitudinal ranges, the pine invariably extending to a lower altitude than the fir. If the fir is equally as resistant as the pine to high temperature, clearly some factor other than surface temperatures must keep the fir from extending downslope into territory occupied by the pine.

It has been demonstrated that in different parts of the ranges of ponderosa pine and Douglas fir the species are represented by races differing in their physiological characteristics, and the same will undoubtedly be found true for other Rocky Mountain trees. Therefore the practice of comparing species representative of the various zones by using seed collected in different parts of the Rockies might be questioned. In northern Idaho at least it would be practically impossible to use seed collected from different zones in the same region unless seed collections were begun several years in advance of the study, for the years when different species produce seed seldom coincide. On the other hand, the consistency of zonal sequences and of all the experimental results obtained in this study provide strong evidence that the procedure followed is justified.

PHASES OF DROUGHT

An unfavorable water balance in plants may be brought about by excessive transpiration, even though soil-moisture conditions are favorable. On the other hand, a degree of desiccation equally as dangerous can result from inadequate soil moisture at times when the transpiration rate is not especially rapid. An attempt has been made to test sepa-

rately the degree of tolerance which conifer seedlings have for these two aspects of drought. Since both atmospheric and soil drought increase in intensity downslope, any differences among the species as to their relative tolerance of these factors might prove significant in differentiating their altitudinal range limits.

Relative tolerance of atmospheric drought

Methods.—The next spring after the temperature tolerance experiments were carried out, another crop of seedlings was grown in the same flat of soil. The species used in this study, together with the seed source, are listed in order of their zonal relationships:

Subalpine fir—San Isabel National Forest, Colorado
Engelmann spruce—San Isabel National Forest, Colorado
Western arborvitae (*Thuja plicata*)—Latah County, Idaho
Douglas fir—Latah County, Idaho
Ponderosa pine—Colorado
Piñon—Colorado

In this experiment the seeds were planted in rows at right angles to the length of the long flat, the rows of mixed species being 5 cm. apart. When the seedlings were 75 days old, drought experiments were begun.

About 24 hours prior to each test the flat was watered heavily, and by the time the test was begun the surface of the sand was dry, although the soil below was well above the field capacity. Desiccation was prevented from extending much below the very surface by covering the soil between the rows of seedlings with strips of glass. After each test, examination showed that this method was adequate to insure favorable level of soil moisture, and therefore any drought effects could be attributed entirely to atmospheric conditions.

During each test a piece of heavy wrapping paper was bent over the flat and secured to both sides with thumbtacks in such a manner that a tunnel, open at both ends, remained above the soil. The diameter of the open ends was adjusted to accommodate an electric desiccating fan, which sent a current of warm dry air through the tunnel across the foliage of the seedlings. Midway during each test the fan was moved from one end of the flat to the other, so that any effects due to differences in the distances between the fan and rows of seedlings would be partly offset, and the results seemed to indicate that desiccation was approximately uniform. An atmometer with the sphere at the end of the tunnel opposite the fan measured the evaporative rates during the tests. These rates were probably a little higher than this instrument recorded, since it was always located at the end of the tunnel farthest from the fan and there the relative humidity was undoubtedly higher than anywhere else along the tunnel, owing to the continual addition of moisture by the air current as it passed through the foliage of the seedlings as well as over the very small percentage of the soil surface not conveniently covered with the glass.

On the first test the seedlings were subjected for 4 hours to an artificial breeze with a mean evaporative rate of 10.3 ml. per hour. It was planned thereafter to make a series of tests at intervals of a few days, each time increasing the duration of treatment, to determine the degree of drought which the different species could endure. Duration tests of 6, 8, and 10 hours were made, during which the mean rates of evaporation were 9.4, 9.9, and 8.5 ml. per hour, respectively. During these tests sufficient measurements of air

temperature were made within the wind tunnel so that it was certain that the temperature remained well below the critical levels as found in the preceding experiment, and the fact that survival was nearly 100 per cent proves that this factor was not important. The fifth test, which lasted for 12 hours, was conducted on a day during which insolation became so exceptionally strong that practically all seedlings succumbed, and there was no assurance but that high temperature played as important a role as the excessively high evaporation rate. However, the duration and intensity of the previous tests had already increased to such a degree that the most extreme conditions of atmospheric drought encountered by these seedlings in their natural habitats had been exceeded, so that the data obtained prior to the final test are sufficient to answer the question which prompted the tests.

Results.—During drought tests of 6–10 hours, very few seedlings succumbed (table 2), and none of these died as a result of the two most severe tests. The survival and vigor of none of the species was impaired by a 10-hour exposure to a current of warm (not critically hot) air which produced a mean evaporative rate of 8.5 ml. per hour.

Evaluation of results.—In all cases the degree of artificial drought maintained in the preceding experiments was extremely severe in comparison with field conditions in the Rocky Mountain region. On a hot midsummer day at Moscow, Idaho, which is situated below lower timberline, the evaporation rate at 20 cm. above dry barren ground in full sunlight has measured but 3 ml. per hour for the 12-hour period centering about noon. The maximum hourly evaporation rate which Caldwell (6) obtained with cylindrical atmometers in the desert near Tucson, far below lower timberline, was only 6.1 ml. per hour. With the lowest mean hourly evaporation rate 8.5 ml. per hour in these experiments, it is apparent that all the coniferous seedlings tested were able to survive atmospheric drought far in excess of what most of the species normally encounter in nature. No importance can therefore be assigned this aspect of the environmental water balance per se in determining the altitude down to which each species can extend its range.

TABLE 2

Comparative abilities of seedlings to endure artificially produced conditions of atmospheric drought

Duration of period (hours)	Mean hourly evaporation (ml.)*	Mortality (%)					
		Pinus edulis	Pinus ponderosa	Pseudotsuga taxifolia	Thuja plicata	Picea engelmannii	Abies lasiocarpa
4	10.3	0	0	0	2	4	4
6	9.4	0	0	2	3	2	0
8	9.9	0	0	0	0	0	0
10	8.5	0	0	0	0	0	0
12	12.7	87	92	81	97	94	96
Total no. of seedlings..		46	50	40	112	47	47

* From standardized spherical atmometer.

Additional data which show the relative insignificance of the evaporative rate, at least in so far as it operates directly on the seedlings, have been obtained by measuring evaporation in five vegetation zones (from the prairie to the Engelmann spruce zone) in the vicinity of Moscow (8). These field data show that there is no important difference in the evaporation rates in climax associations from one zone to the next during the season most critical for seedling survival. This, of course, does not preclude the likelihood that the slight increase in evaporation rate downslope does not have an indirect effect upon seedling survival through its

influence in drying the surface layers of soil.

The conclusion already reached, that —among other species—Douglas fir seedlings are able to withstand a much drier air than they ever encounter within the natural range of the species, appears to be at variance with PEARSON's conclusions (17). In Arizona he found that Douglas fir seedlings succeed in the shelter of aspen cover but not in adjacent forest openings. Soil moisture was favorable in both habitats, whereas the evaporative rate was at times as much as 90 per cent greater in the open, and it was concluded that the evaporative rate was the controlling factor. This suggestion may hold true for seedlings younger than those used in the present drought tests, or possibly some other factor not measured may have differed sufficiently to bring about different mortality rates in the two habitats which PEARSON compared.

RELATIVE TOLERANCE OF SOIL DROUGHT

Obviously, any variation on the part of the seedlings of different species of conifers to endure dry soil might be of considerable significance in setting different lower altitudinal limits, for both the frequency and the intensity of drought periods increase downslope.

METHODS.—A few seeds of each of the species used in the preceding experiment were planted in each of eighteen metal containers 12 cm. in diameter and 8 cm. in depth. These were coated inside with asphaltum and were crowded together on the greenhouse bench so that excessive soil heating through direct insolation upon the outside of the cans was avoided.

Although conifer seedlings quickly become too cutinized to show when the wilting point is reached, it has been demonstrated that the detection of the attainment of the wilting percentage in such plants is easily accomplished in an indirect manner by interplanting species with more delicate leaf structure (9), since the wilting percentage is essentially the same for all species. When the conifer seedlings were 70 days old, sixteen grains of wheat were sown in each pot. By the time the wheat shoots were about 1 dm. tall their extensive and finely divided root systems had spread throughout the soil mass, and at this time irrigation was suspended so that the plants (coniferous and wheat seedlings) thenceforth gradually reduced the soil moisture to the wilting percentage, as indicated by the appearance of the wheat leaves. Through this use of wheat seedlings it was hoped to overcome the problem of achieving soil drought simultaneously for the deep-rooted pines and the shallow-rooted spruce and fir. The soil mass was so thoroughly ramified by the wheat root systems that it seems safe to assume that its moisture content was reduced rather uniformly and the wilting percentage attained at all horizons at approximately the same time.

Note was taken of the time at which most of the wheat leaves in each pot wilted permanently. Several of the pots were allowed to remain with the soil moisture at or below the wilting percentage for 2 days, then they were watered and the seedlings observed for a time to determine the effects, if any, of the short interval of drought on the vigor of the shoots of the various conifer seedlings rooted in the pot. A second group of pots was allowed to remain in the desiccated condition for 4 days, etc., until a series of data for drought periods up to 10 days was accumulated. This experiment was performed twice, with essentially identical results; the more complete set of data is presented in table 3.

RESULTS.—There were definite differences among species with respect to the abilities of their seedlings to survive approximately identical conditions of soil drought. In general, the conclusion seems warranted that those species characteristic of relatively high elevations can tolerate very little desiccation, whereas those which normally grow at low altitudes can survive periods of soil drought several times longer without apparent injury. However, other tests not included in table 3 indicate that even the drought-resistant seedlings of piñon cannot endure dry soil for more than about 12 days.

PEARSON (18) found that the more mesophytic species of conifers "possess a surprising degree of resistance to transpiration when the water supply is reduced to a dangerous level." This characteristic may well explain the greater tolerance of soil drought in these species, in that the ability greatly to decrease water loss postpones the attainment of a fatal degree of protoplasmic dehydration.

EVALUATION OF RESULTS.—In all forest zones in the Rockies, the soil-moisture content is favorable in the spring and early summer, when tree seedlings germinate, but decreases progressively during the summer. Both PEARSON (18) and HAIG *et al.* (11) have emphasized the importance of the rate of elongation of the roots of seedlings with respect to this progressive desiccation from the soil surface downward. Growth water remains available to the seedlings only so long as the distal extremities of their root systems keep ahead of the downward extension of the dry zone. When due consideration is given to the well-known differences among the species as to rate of penetration in depth of their root systems, the significance of the differences in physiology as here reported is greatly enhanced. Those species shown to be most tolerant of dry soil likewise have the greatest rates of root elongation; thus the two characteristics have cumulative value in regard to survival in dry climates. For example, the tap root of piñon, which has the most rapid rate of elongation and consequently runs the least risk of being overtaken by progressive soil desiccation, is at the same time the most able to endure such a dangerous condition until it can be alleviated by precipitation.

TABLE 3

COMPARATIVE SURVIVAL OF SEEDLINGS WHEN SOIL-MOISTURE CONTENT WAS BELOW WILTING COEFFICIENT, 5 DAYS AFTER IRRIGATION HAD BEEN RESUMED

No. of days of soil drought	Mortality (%)					
	Pinus edulis	Pinus ponderosa	Pseudotsuga taxifolia	Thuja plicata	Picea engelmannii	Abies lasiocarpa
2	0	3	0	......	33	0
4	0	0	50	100	50	100
6	0	19	80	100	100	100
8	0	50	100	100	100	100
10	0	100	100	100	100	100

Field studies have invariably shown that the intensity of soil drought increases downslope in the Rockies. WEAVER (26), HANSON (12), and WHITFIELD (27) have presented limited but consistent data which demonstrate this point. PEARSON (19), who has made the most complete series of studies of this factor to date, stated that in the ponderosa-pine zone in the southern Rockies at "depths of 12 inches or less, complete or nearly complete exhaustion of growth water may usually be expected in the latter part of June. All information at hand indicates that far more extended depletion

is the rule in the piñon-juniper type. In the Douglas fir and Engelmann spruce types, droughts during the main growing season must be exceedingly rare." Periodic measurements of soil moisture in the northern Rockies (8) bear out these conclusions. Thus the environmental measurements appear to furnish another source of indirect evidence concerning the dominant role played by the moisture factor, for approximately equal degrees of drought recur season after season in various zones in the southern Rockies, and there appears to be close similarity in the manner with which differing degrees of drought are correlated with the same forest types in the northern and southern Rockies. The importance of moisture as a governing factor in setting lower limits of altitudinal distribution in the Rockies is still no more than a theory, but there has accumulated an impressive body of field data and physiological tests to substantiate it.

Summary

1. The differing abilities displayed by various Rocky Mountain coniferous trees to extend their ranges downslope into altitudes of increasingly hotter and drier climate has been attributed by some investigators to corresponding differences in the ability of their seedlings to tolerate heat, while others explain the same phenomenon on the basis of drought. In an effort to determine which of these is correct, greenhouse studies were made in which seedlings of trees with different altitudinal ranges have been compared in respect to their tolerance of high soil-surface temperatures and their tolerance of atmospheric and soil drought.

2. In general, it was found that the lower the altitudinal distribution of a species the greater the tolerance of its seedlings for high soil-surface temperatures. However, this factor apparently cannot explain the lower altitudinal limits, since (*a*) the maximum temperature level which each species can tolerate well exceeds the maximum temperatures obtaining in numerous micro-habitats below its altitudinal range; (*b*) a number of species, including subalpine fir, can be grown far below their natural lower limits without making compensation for the higher soil-surface temperatures there; and (*c*) the correlation between altitudinal distribution and temperature tolerance is not absolute, ponderosa pine having no greater temperature tolerance than Douglas fir, although it extends to distinctly lower altitudes.

3. When supplied with abundant soil moisture, even the subalpine trees can tolerate an intensity of atmospheric drought far in excess of that to which they are subjected under natural conditions. Furthermore, all species, regardless of altitudinal range, have approximately equal resistance to atmospheric drought. This aspect of drought therefore is apparently of no direct importance in determining the lower limits of the trees.

4. A distinct variation exists in the length of time over which the seedlings of different species can tolerate a lack of growth water; in general, the lower the altitudinal range the longer the period of soil drought which can be endured. Since all researches to date have indicated that the intensity and duration of drought increase downslope, this factor appears to offer at least a partial explanation of the differences in lower range limits. The significance of this difference among the species to endure soil drought is greatly magnified by the fact that the species of low altitude have the most rapid rates of root penetration. Not only are they bet-

ter able to endure soil drought, but they are more likely to escape its influence.

5. The accuracy with which uniformity of environmental conditions can be maintained in different parts of the same culture varies inversely with the number of experimental plants used, and therefore conclusions based upon a few carefully tended plants are at least equally as valuable as greater numbers of plants but with conditions less uniform. In the temperature tolerance tests in which the fewest numbers of seedlings were used, the same conclusions may be drawn from either half of the lot of seedlings.

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LITERATURE CITED

1. BATES, C. G., The transect of a mountain valley. Ecol. 4:54–62. 1923.
2. ———, Physiological requirements of Rocky Mountain trees. Jour. Agr. Res. 24:97–164. 1923.
3. ———, Some relations of plant ecology to sylvicultural practice. Ecol. 7:469–480. 1926.
4. BATES, C. G., and ROESER, J. JR., Relative resistance of tree seedlings to excessive heat. U.S.D.A. Bull. 1263. 1924.
5. BLUMER, J. C., A comparison between two mountain sides. Plant World 13:134–140. 1910.
6. CALDWELL, J. S., The relation of environmental conditions to the phenomenon of permanent wilting in plants. Physiol. Res. 1:1–56. 1913.
7. DAUBENMIRE, R. F., Merriam's life zones. Quart. Rev. Biol. 12:327–332. 1939.
8. ———, The distribution and ecology of climax forest zones on the Thatuna Range in northern Idaho. Unpublished. 1943.
9. DAUBENMIRE, R. F., and CHARTER, H., Behavior of woody desert legumes at the wilting percentage of the soil. BOT. GAZ. 103:762–770. 1942.
10. GRAHAM, E. H., Botanical studies in the Uintah Basin of Utah and Colorado. Carn. Mus. Ann. 26:1–432. 1937.
11. HAIG, I., *et al.*, Natural regeneration in the western white pine type. U.S.D.A. Tech. Bull. 767. 1941.
12. HANSON, H. C., A study of the vegetation of northeastern Arizona. Univ. Neb. Studies 24: 85–175. 1924.
13. HOFMANN, J. V., Natural regeneration of Douglas fir in the Pacific Northwest. U.S.D.A. Bull. 1200. 1924.
14. ISAAC, L. A., Seedling survival on burned and unburned surfaces. Pacific Northw. For. Range Exp. Sta. Res. Note 3:3–4. 1929.
15. KORSTIAN, C. F., and FETHEROLF, N. J., Control of stem girdle of spruce transplants caused by excessive heat. Phytopath. 11:485–490. 1921.
16. LARSEN, J. A., Forest types in the northern Rocky Mountains and their climatic controls. Ecol. 11:631–672. 1930.
17. PEARSON, G. A., The role of aspen in the reforestation of mountain burns in Arizona and New Mexico. Plant World 17:249–260. 1914.
18. ———, Studies in transpiration of coniferous tree seedlings. Ecol. 5:340–347. 1924.
19. ———, Forest types in the southwest as determined by climate and soil. U.S.D.A. Tech. Bull. 247. 1931.
20. PIERSON, R. K., Personal correspondence. 1942.
21. ROESER, J., JR., Transpiration capacity of coniferous seedlings and the problem of heat injury. Jour. For. 30:381–395. 1932.
22. SHAW, C. H., The vegetation of the Selkirks. BOT. GAZ. 61:477–494. 1916.
23. SHREVE, F., The vegetation of a desert mountain range as conditioned by climatic factors. Carn. Inst. Wash. Publ. 217. 1915.
24. TOUMEY, J. W., and NEETHLING, E. J., Insolation a factor in the natural regeneration of certain conifers. Yale Univ. Sch. For. Bull. 11. 1924.
25. VESTAL, A. G., Foothills vegetation in the Colorado Front Range. BOT. GAZ. 44:353–385. 1917.
26. WEAVER, J. E., A study of the vegetation of southeastern Washington and adjacent Idaho. Univ. Neb. Studies 17:1–114. 1917.
27. WHITFIELD, C. J., The ecology of the vegetation of the Pike's Peak region. Ecol. Monog. 3:75–105. 1933.

EFFECTS OF NUTRIENT, PHOTOPERIOD, AND NIGHT TEMPERATURE ON THE DEVELOPMENT OF GUAYULE SEEDS

M. R. WHITEHEAD[1] AND JOHN W. MITCHELL[2]

Introduction

Although the rubber-producing plant guayule, *Parthenium argentatum*, produces abundant seeds when grown under favorable conditions, a relatively large number of these contain immature embryos. Improvement in the quality of the seeds produced would be of value from a practical standpoint, since the number gathered could be reduced without diminishing the yield; and from the standpoint of an experimental breeding program, it would make possible the more rapid multiplication of desirable strains.

Experiments were undertaken to test the effect of several environmental factors on the quantity and quality of the seeds, as well as on the amount of vegetative growth and rubber produced by the plant. The results reported here concern seed production when the plants were (*a*) supplied with various nutrients, (*b*) subjected to different night temperatures, and (*c*) grown under different photoperiods.

Investigation

Nutrient supply

METHODS.—At the time the present experiments were undertaken, relatively little work had been done with respect to the nutrient requirements of guayule. Preliminary efforts were therefore directed toward a general study of the effect of different types of nutrient solutions on growth of the plant and its rate of seed production, rather than toward a detailed study of the influence of any one specific element.

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Two series of solutions were used, as described by HAMNER (4). In the first series the cations Ca, K, and Mg varied in concentration while the anions remained essentially constant, and in the second series the anions N, P, and S varied while the cations remained constant. Since the object was to select a nutrient solution which would result in the greatest amount of growth, together with a maximum rate of seed and rubber production, it was not considered necessary to test solutions which did not contain all the essential elements.

Three stock solutions were used to prepare nutrient solutions in which the anions varied. Stock solution A consisted of 0.0037 mol. KNO_3, 0.0037 mol. $Mg(NO_3)_2$, and 0.0049 mol. $Ca(NO_3)_2$. Solution B consisted of 0.0037 mol. K_2SO_4, 0.0048 $CaSO_4$, and 0.0037 mol. $MgSO_4$. Solution C consisted of 0.003 mol. $Ca(H_2PO_4)_2$, 0.0023 mol. $MgHPO_4$, and 0.0037 mol. KH_2PO_4. Sufficient phosphate was added as H_3PO_4 to make the phosphate ion in solution C equivalent to that in a 0.0037 mol. solution of $MgHPO_4$.

In a similar manner three stock solutions were used to prepare nutrients which varied with respect to the cations. Stock solution D contained 0.0037 mol. $Mg(NO_3)_2$, 0.0037 mol. $MgSO_4$, and 0.0023 mol. $MgHPO_4$. Phosphate was added as before to raise the concentration of this ion in D to that of a 0.0037 mol. solution of $MgHPO_4$. Solution E contained 0.0037 mol. KNO_3, 0.0037 K_2SO_4, and 0.0037 KH_2PO_4. Solution F contained 0.0049 mol. $Ca(NO_3)_2$, 0.0049 mol. $CaSO_4$, and 0.0033 mol. $Ca(HPO_4)_2$. All solutions were made up in tap water.

By adding together the required

amounts of these stock solutions, it was possible to make nutrient solutions which varied with respect to their anion or cation content, while the ionic concentration of the cations remained essentially the same (table 1). However, when the concentrations of the elements nitrogen, phosphorus, and sulphur were expressed on a part-per-million basis, they necessarily varied from 78 to 118, 131 to 175, and 124 to 144 p.p.m., respectively. It was likewise possible to make a corresponding series of solutions which varied a desired amount with respect to their cation content, while the anion concentration remained essentially constant. Expressed on a part-per-million basis, calcium, magnesium, and potassium in the latter series varied from 154 to 184, 66 to 84, and 168 to 240 p.p.m., respectively. Boron, zinc, copper, and manganese were added at the rate of 0.5 p.p.m. in the form of boric acid, zinc chloride, copper chloride, and manganese chloride. Iron was added as ferric citrate at the rate of 0.3 p.p.m.

One thousand uniform transplants, variety 593, which had been grown in a nursery in California, were selected for size and uniformity. These plants were of a size commonly used for field planting. They were planted individually, during the first week of July, in a gravel-sand mixture contained in glazed earthenware crocks of 2- and 3-gallon capacity. The mixture contained 27% by weight of coarse sand which passed an $\frac{1}{8}$-inch screen, 31% gravel which passed a $\frac{1}{4}$-inch screen, and 42% gravel which failed to pass through a $\frac{1}{4}$-inch screen. The crocks were rounded on the bottom and provided with a 1-inch hole for drainage. This opening was covered with a piece of bronze screen to prevent the loss of sand and gravel. The crocks were supported on bricks so that they were approximately 2 inches above the ground, or above the surface of gravel on a bench in the case of the greenhouse experiments. This allowed free drainage and prevented the roots from growing out of the pots. Sufficient nutrient solution was applied to flush the pots three times weekly. The sand-gravel mixture was kept moist during the intervening time by light applications of tap water, which were applied carefully so as not to flush the nutrients from the crocks.

TABLE 1

PROPORTIONS OF STOCK SOLUTIONS USED IN PREPARATION OF NUTRIENT SOLUTIONS

SOLUTION	STOCK SOLUTIONS			SOLUTION	STOCK SOLUTIONS		
	A	B	C		D	E	F
1	$\frac{2}{3}$	$\frac{1}{6}$	$\frac{1}{6}$	11	$\frac{2}{3}$	$\frac{1}{6}$	$\frac{1}{6}$
2	$\frac{1}{2}$	$\frac{1}{6}$	$\frac{1}{3}$	12	$\frac{1}{2}$	$\frac{1}{6}$	$\frac{1}{3}$
3	$\frac{1}{3}$	$\frac{1}{6}$	$\frac{1}{2}$	13	$\frac{1}{3}$	$\frac{1}{6}$	$\frac{1}{2}$
4	$\frac{1}{6}$	$\frac{1}{6}$	$\frac{2}{3}$	14	$\frac{1}{6}$	$\frac{1}{6}$	$\frac{2}{3}$
5	$\frac{1}{2}$	$\frac{1}{3}$	$\frac{1}{6}$	15	$\frac{1}{2}$	$\frac{1}{3}$	$\frac{1}{6}$
6	$\frac{1}{3}$	$\frac{1}{3}$	$\frac{1}{3}$	16	$\frac{1}{3}$	$\frac{1}{3}$	$\frac{1}{3}$
7	$\frac{1}{6}$	$\frac{1}{3}$	$\frac{1}{2}$	17	$\frac{1}{6}$	$\frac{1}{3}$	$\frac{1}{2}$
8	$\frac{1}{3}$	$\frac{1}{2}$	$\frac{1}{6}$	18	$\frac{1}{3}$	$\frac{1}{2}$	$\frac{1}{6}$
9	$\frac{1}{6}$	$\frac{1}{2}$	$\frac{1}{3}$	19	$\frac{1}{6}$	$\frac{1}{2}$	$\frac{1}{3}$
10	$\frac{1}{6}$	$\frac{2}{3}$	$\frac{1}{6}$	20	$\frac{1}{6}$	$\frac{2}{3}$	$\frac{1}{6}$

The seedlings were divided into two groups of equal numbers. One group was placed outdoors during July, August, September, and the first half of October, so that the plants were exposed to direct light and prevailing temperatures during this period. The remaining group was placed in a greenhouse. In both groups treatments were arranged as randomized blocks. Seven replications were used in the case of the greenhouse experiment and five in the case of the experiment conducted outside.

Plants grown in the open were subjected to direct light which reached an intensity of more than 10,000 foot-candles on very clear days, as measured

by a Weston light meter. Night temperatures during July and August varied between 60° and 75°, while day temperatures sometimes reached 90°–98° F. During September and early October night temperatures fell as low as 30°–40°, and day temperatures seldom were more than 70°–80°. In October, those plants which had grown outside during the summer were moved into the greenhouse, where they were subjected during the fall and winter months to a day temperature of 65°–75° and a night temperature of 45°–55° F.

Plants grown in the greenhouse were subjected to light which filtered through double-strength glass. The intensity was reduced by approximately 30% below that of direct light, as measured by a Weston light meter. Night temperatures in the greenhouse during July and August seldom fell below 70°, and day temperatures usually ranged between 85° and 95° and occasionally reached 105°–110° F. During September and early October, day temperatures in the greenhouse still ranged 5°–15° above outside temperatures, although night temperatures were more nearly the same. The plants which had been subjected to relatively high temperatures under greenhouse conditions during the summer months were also grown at relatively high temperature levels during the fall and winter months; the day temperatures were 70°–80° and the night temperatures 60°–70° F.

The plants began to flower during the latter part of July, and the first seed collections were made the middle of August. The flowering season continued during the remainder of August and all of September but became greatly reduced with the onset of cooler weather during October. Practically no flowers developed after the middle of October.

In collecting samples, seeds were gathered at intervals of 2–5 days, when ripe. They were separated from the chaff and stored air-dry at room temperature in groups, which in the end represented the total number of seeds produced during the summer and fall seasons by the respective replications of each treatment.

The expression "seed yield" is used here to mean the dry weight of the total number of seeds produced by a plant during a given period of time. The seed, pistillate floral parts, and attached disk flowers (2) were included in the seed weights because of difficulty of separation. Two methods of measurement were employed to determine the quality of the seeds produced. The one most generally used consisted of determining the percentage of seeds within a given lot which could be classified as heavy, and which for the most part contained seeds with mature embryos. The other method consisted of testing the heavy seeds for germination.

Relative to estimating the percentage of heavy seed, a satisfactory and rapid separation of those with mature from those with immature embryos was accomplished by means of the specific gravity method, using Skellysolve[3] fraction F (fig. 1). The relatively heavy seeds included most of those with mature embryos, while the relatively light seeds most of those with immature embryos.

For the purpose of measuring percentage germination, the light seeds were discarded and only the heavy ones tested. These were first soaked in tap water overnight at room temperature. The water was then drained off and a 4 per cent mixture of sodium hypochlorite

[3] Miss Alice M. Andersen, Bureau of Food Distribution Administration, first used Skellysolve to separate guayule seeds.

added for a period of 20 minutes. The seeds were then washed with water two to three times to remove the hypochlorite. They were then placed on moist filter paper in petri dishes, and these were alternately placed at 20° C. for 16 hours and then at 30° C. for 8 hours. The dishes were kept in continuous darkness, except when being transferred from one temperature to the other.

Germination was considered to be that stage of development at which the radical emerged from the seed coat. The rate of growth subsequent to this stage was variable, as some plants grew vigorously while others grew more slowly. During the first 2–3 days' growth the seedlings were classified as good or poor, depending on the rate at which the radical was extended. In this way it was possible to measure the percentage of seedlings which grew vigorously during the very early stages of development.

Two hundred and twenty-five seeds were tested for germination in the case of the majority of the nutrient treatments. Plants of a few treatments failed to produce sufficient seeds for adequate germination tests and therefore a statistical analysis of germination data was not attempted.

Results.—Figure 2 compares the amount and quality of seeds produced by plants grown on solutions varying with respect to anions. Plants supplied with a relatively high level of nitrogen (solution 1) produced approximately nine times as much total yield of seeds by weight as the average amount produced by plants supplied with relatively low levels of nitrogen (solutions 4, 7, 9, 10). In general, an increase in the amount of nitrogen above 48 p.p.m. was associated with increase in the weight of seeds produced, irrespective of variations in the sulphur and phosphorus content of the solutions. Thus plants watered with solutions 4, 7, 9, and 10, which all contained a low level of nitrogen, produced relatively few seeds. No significant differences in seed production were noted between plants watered with these four solutions, although the concentration of sulphur varied from 65 to 260 p.p.m. and that of phosphorus from 72 to 288 p.p.m. Solutions 3, 6, and 8 contained a somewhat higher concentration of the nitrate ion, and the plants watered with them produced a significantly greater number of seeds than did those of the previous series. Again there was no significant difference in weight of seed produced due to the different levels of sulphur and phosphorus used in this second series. The use of solutions 2 and 5 resulted in a further increase in seed production, and the increase was associated with an increase in the concentration of nitrate ion used. As in the previous series, varia-

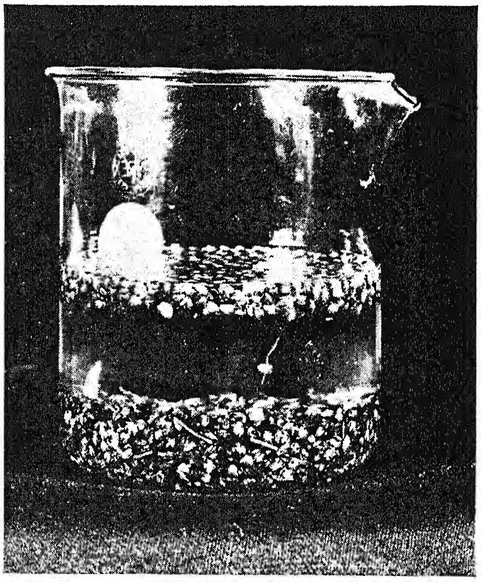
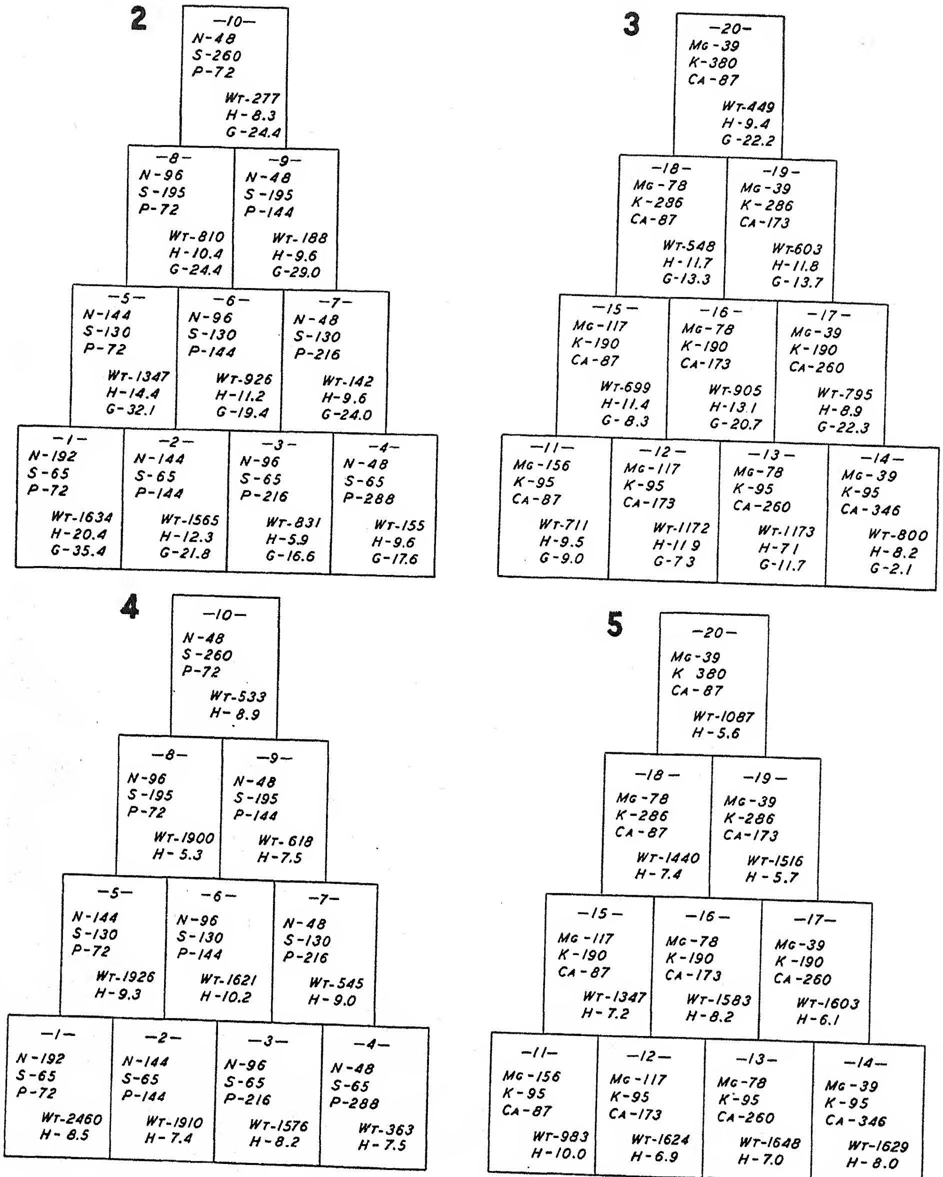


Fig. 1.—Method of separating light from heavy seeds, using Skellysolve.



FIGS. 2–5.—Seed production as influenced by nutrient solutions: Figs. 2, 3, plants grown outdoors; figs. 4, 5, grown in greenhouse. (*wt*, average weight of seeds collected during 2 months from each replication, four plants; 236 mg. difference required for statistical significance in seed weight of 1:19 in figs. 2 and 3. *H*, percentage of heavy seeds; difference of 5.5 per cent required for statistical significance of 1:19. *G*, percentage germination of heavy seeds.) Figs. 2, 4, solution varied with respect to N, nitrogen; S, sulphur; P, phosphorus; figs. 3, 5, with respect to Mg, magnesium; K, potassium; Ca, calcium.

tions in the amount of sulphur and phosphorus were not associated with significant variations in the weight of seeds produced. From this it appears that the range of concentrations in the case of the sulphate and phosphate ions was not critical, but that an increase from 48 to 192 p.p.m. of nitrogen in the nutrient was associated with a marked increase in the amount of seeds.

Essentially the same type of response was observed with respect to the quality of seeds produced by plants grown on solutions containing different amounts of the anions. An increase in nitrogen from the lowest level (solutions 4, 7, 9, 10) to the highest (solution 1) was associated with substantial increase in the percentage of seeds with mature embryos, that is, the percentage of heavy seeds. Seeds from plants grown on a solution containing a relatively large amount of nitrogen also gave the highest percentage germination. The concentrations of sulphur and phosphorus used were not critical with respect to seed quality.

Figure 3 shows the effect of different concentrations of cations on the amount of seed produced. A solution containing a relatively low concentration of magnesium and calcium and a high level of potassium (solution 20) did not favor seed production. With increasing amounts of magnesium and calcium there was a corresponding increase in the amount of seed, which reached a maximum in the case of plants watered with solutions containing 78–117 p.p.m. of magnesium and 173–260 p.p.m. of calcium (solutions 12 and 13). In these solutions potassium was at the lower limit of the concentration range for this element, but at this level there was apparently an ample supply of potassium. A high level of calcium together with a low level of magnesium was associated with reduced seed production (solution 14). The reverse of this combination (high magnesium and low calcium) was also associated with reduced seed production (solution 11), and plants grown on this solution showed symptoms of injury which were apparent mainly in the leaves.

A relatively high percentage of heavy seeds was produced by plants supplied with a moderate amount of magnesium and calcium and a relatively high level of potassium (solution 16), although a somewhat greater total weight of seeds was obtained through the use of solutions containing essentially the same levels of magnesium and calcium but a lower level of potassium.

When different concentrations of anions were supplied to plants grown under greenhouse conditions (fig. 4), the responses with respect to the amount of seeds produced were similar to those of plants treated in the same way but grown in the open. Increasing amounts of nitrogen supplied to the plants, 48–192 p.p.m., resulted in a corresponding increase in the weight of seeds produced (cf. 4, 7, 9, 10 with solution 1). A solution which contained a low sulphur level, together with low nitrogen and high phosphorus, was somewhat less favorable than were other solutions low in nitrogen but containing somewhat more sulphur (cf. solution 4 with 7, 9, and 10). In general, the concentrations of sulphur and phosphorus were less effective in altering seed production than were the concentrations of nitrogen.

Considering the utilization of nitrogen, plants grown outside and supplied with 192 p.p.m. of nitrogen produced approximately nine times more seeds than did other plants given solutions containing 48 p.p.m. of nitrogen (cf. solution 1 with the average of 4, 7, 9,

10; fig. 2). In contrast, plants grown in the greenhouse and given 192 p.p.m. of nitrogen produced only 4.8 times more seeds than did others watered with solutions containing 48 p.p.m. of nitrogen (fig. 4).

Under greenhouse conditions, as in the case of plants grown outside, those that received the highest level of magnesium together with a low level of calcium and potassium (solution 11) showed symptoms of injury, especially in the leaves, and these plants produced relatively few seeds (fig. 5).

Greenhouse plants responded somewhat differently from those grown outside, in that their rate of seed production was not inhibited by application of a solution containing 39 p.p.m. magnesium and a relatively high level of calcium. Also, plants grown under the reduced intensity of light and relatively high temperatures prevailing in the greenhouse produced a greater total weight of seeds in the case of most treatments than did those grown outdoors; but they were of poor quality with respect to the percentage containing mature embryos.

Although the nutrient treatments had an effect on the percentage germination of seeds from plants grown outdoors, they did not obviously affect the rate of growth of the seedlings during a 2-week period subsequent to germination. Since no significant differences between treatments were noted in the number of vigorously growing seedlings, data from these observations are not presented.

Photoperiod

Methods.—The object of this experiment was to observe the effect of the length of the daily period of illumination on the number and quality of seeds produced. In general, seedlings were exposed to daily periods of illumination of different lengths, the photoperiodic treatment being applied as soon after germination as feasible. The effect on flower initiation and development and on seed production was observed during several months of treatment and for some time thereafter.

Seeds of variety 406 were germinated in petri dishes as previously described. When the radicals were approximately $\frac{1}{4}$ inch long, selected seedlings were planted in soil and grown for 2 weeks under conditions prevailing in the greenhouse during June. At the end of this time the tops extended about 1 inch above the surface of the soil. Ninety uniform plants were selected and divided into six groups of equal numbers, and each group was placed on a truck which could be moved from the natural light of the greenhouse to rooms where artificial light was the only source of illumination. The number of plants used was limited by the capacity of the trucks.

Photoperiodic treatments were chosen which corresponded in length to natural daily periods of illumination prevailing in different latitudes in the United States suited to the growing of guayule. In addition, two other treatments were added. In one, the plants were subjected to day lengths prevailing at Beltsville, Maryland, during the period between July and January; in the other, supplementary light was added so that the plants were exposed to a continuous period of illumination.

For the purpose of comparing some of the treatments, it was thought desirable to subject them to the same amount of natural light, namely, a basic period of 10 hours, so that the plants would have the same opportunity to synthesize food material. To extend the daily periods of illumination, this basic period was supplemented by means of light from a

white fluorescent lamp. The supplementary light was of low intensity (20 foot-candles) so as to induce a photoperiodic response without bringing about an appreciable amount of photosynthesis.

Treatments were applied from July 1 until November 3, when the plants were removed from the trucks and placed on a greenhouse bench where they all received natural illumination under the same conditions. Photoperiodic responses were observed from September to January, during a major part of which time the plants were in bloom.

RESULTS.—Under the conditions used, a relatively long daily period of illumination favored the initiation of flowers, the production of seeds, and to some extent lengthened the flowering season (table 2). The addition of 3–5 hours of supplemental light to a 10-hour photoperiod of natural light resulted in marked improvement in the quality as well as in the quantity of seeds produced. Plants grown under continuous illumination produced a relatively large number of seeds, but these were of poorer quality with respect to the percentage of heavy seeds. However, plants grown under the influence of the most favorable photoperiodic treatments produced fewer flowers during the fall and winter months than did untreated plants of comparable size grown outside in the natural light prevailing during the spring and summer.

The effect of the photoperiodic treatment was not lasting, since flower primordia were not initiated during January after the long-day treatments had been discontinued in November. Supplementing natural light with light of low intensity from an artificial source had no apparent effect on the vegetative growth under the conditions used.

In general, plants grown under ordinary greenhouse conditions without photoperiodic treatments began to produce flowers in April or early May, when the natural day length was approaching 12 hours, and continued to flower and grow vegetatively during the longer days of summer until the latter part of September, when the days were again less than 12 hours in length. During the short days of fall and winter, vegetative growth was somewhat slower, even at moderate temperatures, than during the summer months, and no appreciable number of flower primordia were ini-

TABLE 2

EFFECT OF PHOTOPERIODIC TREATMENTS ON FLOWER INITIATION AND SEED PRODUCTION BY GUAYULE

TREATMENT	HOURS OF LIGHT			WEIGHT OF SEED PRODUCED, SEPT.–DEC. (MG.)	HEAVY SEED (%)	NO. OF PLANTS THAT FLOWERED (NOV. AND DEC.)	NO. OF PLANTS THAT FLOWERED (JANUARY)	NO. OF FLOWER PRIMORDIA (JANUARY)*	NO. OF PLANTS TREATED
	Natural	Artificial	Total per day						
1	10	0	10	13	0	0	0	0	15
2	10	3	13	287	23.7	6	0	0	15
3	10	5	15	388	22.1	5	0	0	15
4	10	14	24	231	7.0	12	0	0	15
5	14.75–9.5†	0	14.75–9.5	212	16.7	0	0	0	15

* Determined by dissecting branches with aid of microscope.

† Received natural light prevailing from July 1, 1942 to Jan. 15, 1943.

tiated until the onset of vegetative growth during the following spring.

Night temperature

METHODS.—Plants of variety 593, comparable with those generally used for field plantings, were obtained from a nursery in California. Fifty-four of these were selected for size and uniformity and planted in composted soil contained in 8-inch clay pots. The plants were placed on six trucks in groups of equal numbers, so that they could be transported into rooms in which specific temperatures were maintained. The number of plants in this experiment was also limited by the capacity of the equipment available.

During the summer months the plants were moved outdoors from the respective temperature-control rooms each day at approximately 6:00 A.M. and grown under the prevailing temperature and light conditions, then they were returned to controlled temperatures and kept in darkness from approximately 7:00 P.M. until the following morning, when the procedure was repeated. In October and during the following fall and winter months the same procedure was followed, except that the plants were moved each day from the respective temperatures to a greenhouse at approximately 8:00 A.M. All the plants remained at 65°–75° F. until about 6:00 P.M., when they were again returned to the respective control rooms. The plants were repotted several times during the experiment to maintain soil fertility.

RESULTS.—The plants grew vigorously during July, August, and early September. Some plants that received 80°–90° night temperature flowered as late as February, while those exposed to lower night temperatures failed to bloom after the latter part of October. Seed collections were made as the seeds ripened during the period from August to November. The 80°–90° night-temperature treatment resulted in reduced growth, as indicated by the dry weight of the plants and the relatively few seeds, which were of poor quality with respect to the percentage of heavy ones (table 3). Plants grown with a night temperature of 65°–75° produced approximately the same number of seeds but of better quality. Plants grown at the lowest night temperature, 50°–60° F., produced more than three times as many seeds as did those grown at the two higher temperatures, and these were of relatively good quality.

TABLE 3

EFFECT OF NIGHT TEMPERATURE ON VEGETATIVE GROWTH AND AMOUNT AND QUALITY OF SEED PRODUCED

Temperature (° F.)	Average dry weight per plant (gm.)	Total weight of seeds (gm.)	Heavy seeds (%)
50–60	37.5	16.1	25
65–75	36.1	5.1	27
80–90	23.8	5.0	10

Discussion

There is evidence that certain minerals can be mobilized within unripe fruit and seeds at the expense of the vegetative portion of the plant. Thus phosphorus was found to accumulate readily in the green fruit and seeds of tomato at the expense of phosphorus contained in the leaves, when the plants were grown on a limited supply of this element (1). Since under certain conditions developing seeds can accumulate some elements at the expense of the rest of the plant, it may be reasoned that in such instances only wide variations from the optimum nutrient supply would be

reflected in the quantity or quality of the seeds.

In the present experiments, however, guayule was found to be sensitive to the nitrogen supply, and it was not necessary to subject the plant to wide variations in the amounts available in order to influence the amount and quality of the seeds. PULTZ (6) likewise reported that the seed yield of sugar beets was greatly increased as the result of liberal applications of nitrogen, but the treatment apparently had no effect on their quality. BURTON (3) found that the application of potassium and phosphorus fertilizers to Bermuda grass resulted in increased numbers of flower heads. He also observed that nitrogen fertilization resulted in an increase in the number of heads formed on some of the grasses tested, but that seed set was not influenced by the fertilizer treatment. TOLMAN (7) found that the number of small seeds produced by sugar beets increased when nitrogen was added as a fertilizer, but this response was not obtained when both nitrogen and phosphate were added. The percentage germination of seeds was relatively high when nitrogen was added during years favorable to growth, but the addition of nitrogen during unfavorable years decreased the percentage germination.

In the present experiments also, the proportion of calcium and magnesium supplied to the plants influenced the quantity and quality of the seeds. Although the potassium, phosphorus, and sulphur was varied over wide range concentrations, these were apparently within a luxury range, which possibly accounted for the fact that they did not influence seed production under these conditions.

The initiation of flower primordia and the development of seeds were favored to some extent by exposing the plants to relatively long daily periods of illumination, and this effect appeared to be largely due to the action of the long photoperiod on some mechanism other than the process of carbon assimilation, since the response was obtained through the use of supplementary light of low intensity. On the basis of the results observed, however, guayule appears to be less sensitive to variations in the length of the daily period of illumination than are some other plants, such as certain varieties of soybean or chrysanthemum (5). It is evident that guayule is capable of producing flowers in abundance over a range of photoperiods, and that the maintenance of an optimum day length is not of vital importance in connection with seed production.

The blooming period was lengthened to some extent by exposing the plants to a relatively high night temperature, but this extension of the flowering season was not associated with a high rate of seed production, nor the production of seeds of good quality with respect to the number that developed mature embryos. These plants also made less vigorous vegetative growth. It is suggested that these responses to a high night temperature may have resulted from a high rate of respiration during periods of darkness, which would tend to deplete the plant of some materials essential for vigorous vegetative growth and seed development.

Although the direct cause of the prevalence of seeds with immature embryos is not known, these experiments indicate that the condition is more prevalent when the plant is exposed to an environment which fails to supply sufficient amounts of certain inorganic elements, particularly nitrogen, adequate photoperiod, or which does not favor the full utilization of organic materials for the

growth and development of the plant as a whole, because of relatively high night temperature.

Summary

1. In nutrient-culture experiments, the amount and quality of seeds produced by guayule was influenced by some of the nutrient elements made available to the plants. Most seeds were produced when the plants were watered with solutions containing the highest concentration of nitrogen tested, 192 p.p.m. Concentrations of sulphur were varied between 65 and 260 p.p.m., and of phosphorus between 72 and 288 p.p.m., with no apparent effect on the amount or quality of the seeds. Potassium was varied between 95 and 380 p.p.m. with no apparent effect on seed production. With respect to magnesium and calcium, the two most favorable solutions contained 78 and 117 p.p.m. magnesium and 173 and 260 p.p.m. calcium.

2. Plants were induced to bloom during the winter months by supplementing a 10-hour period of daylight with 3–14 hours of light from an artificial source. When the long-day treatment was discontinued in November, flower production decreased during December and the plants failed to bloom or initiate flower primordia during January.

3. Plants subjected to a day temperature of 65°–75° and a night temperature of 80°–90° F. initiated flowers during the winter months although the natural daily period of illumination was not extended by means of light from an artificial source. In contrast, plants exposed to the same temperatures during the day, and night temperatures of 65°–75° or 45°–55° F., failed to flower during the winter months.

4. Plants grown under conditions of relatively warm days and cool nights (45°–55° F.) produced more seeds of good quality. Plants exposed to moderate night temperatures (65°–75°) produced fewer seeds, which were also of good quality. Plants subjected to relatively high night temperatures (80°–90°) produced approximately the same number of seeds as those exposed to a moderate night temperature, but these were of relatively poor quality.

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Beltsville, Maryland

LITERATURE CITED

1. Arnon, D. I., Stout, P. R., and Sipos, F., Radioactive phosphorus as an indicator of phosphorus absorption of tomato fruits at various stages of development. Amer. Jour. Bot. 27:791–798. 1940.
2. Artschwager, E., Contribution to the morphology and anatomy of guayule (*Parthenium argentatum*). U.S. Dept. Agr. Tech. Bull. 842. 1943.
3. Burton, G. W., Factors influencing seed setting in several southern grasses. Jour. Amer. Soc. Agron. 35:465–474. 1943.
4. Hamner, C. L., Growth responses of Biloxi soybeans to variations in relative concentrations of phosphate and nitrate in the nutrient solution. Bot. Gaz. 101:637–648. 1940.
5. Parker, M. W., and Borthwick, H. A., Length of day and crop yields. U.S.D.A. Misc. Publ. 507. 1942.
6. Pultz, L. M., Relation of nitrogen to yield of sugar beet seed and accompanying changes in composition of roots. Jour. Agr. Res. 54:639–654. 1937.
7. Tolman, B., Sugar beet seed production in southern Utah, with special reference to factors affecting yield and reproductive development. U.S.D.A. Tech. Bull. 848. 1943.

HISTOLOGICAL STUDIES ON PARTHENOCARPIC FRUITS OF LILIUM REGALE INDUCED BY GROWTH SUBSTANCES[1]

CONTRIBUTIONS FROM THE HULL BOTANICAL LABORATORY 553

J. M. BEAL

Since GUSTAFSON (2) first reported the production of mature seedless fruits through the application of lanolin mixtures of indoleacetic, indolepropionic, indolebutyric, and phenylacetic acids to the styles of certain solanaceous plants, considerable additional work has been done on a variety of species. A summary of these investigations has recently been presented by the same writer (3).

The only instance in which a detailed histological study of induced parthenocarpic fruits in comparison with ones developed following pollination is that of GARDNER and KRAUS (1). They found that development of parthenocarpic fruits in holly following spraying with indoleacetic acid paralleled almost precisely that following pollination, except that cells of the stigmas of sprayed fruits proliferated more than those pollinated, did not collapse and suberize so quickly, and there was a complete lack of embryo or endosperm development in the sprayed fruits. No marked or disorderly cell proliferation occurred in the pistils to which the acid had been applied. The parthenocarpic berries averaged as large and were as turgid and green as those following pollination, and at the time of fruit ripening they showed no differences in color.

Material and methods

During the spring and early summer of 1942, ovaries on potted plants of *Lilium regale* growing in the greenhouse were treated with 1 per cent lanolin mixtures of indoleacetic, naphthaleneacetic, and naphthoxyacetic acids and with a combination consisting of equal volumes of the three 1 per cent concentrations stirred thoroughly together before using.

Before treating, the flowers were allowed to open. The stamens were then pulled off and the stigma and style removed by cutting squarely across the top of the ovary at the base of the style. The cut surface of the ovary was then covered with one of the lanolin mixtures and left for varying lengths of time. Controls were handled in the same manner except they were treated with lanolin alone or, in a few cases, were given no further treatment after removal of the style.

In none of the controls did further marked growth occur despite the occurrence of mitotic figures in carpel walls, integuments, and nucellus at the time of treatment. There was relatively slight difference in subsequent behavior of the controls, whether treated with lanolin only or given no treatment other than removal of the style. Those treated with lanolin alone dried and shriveled perhaps a little less slowly, but none enlarged to more than one and one-third their diameter at the time of treatment.

The ovaries were collected at various intervals following treatment. They were cut transversely into segments 2–4 mm. in length, fixed in Navashin's solution, and imbedded in paraffin. Sections were cut at 10 μ and stained in Flemming's triple stain.

Gross responses

Ovaries to which the three growth substances and the combination were applied all enlarged in length and diameter at approximately the same rate and attained nearly the same final dimensions

[1] This work was aided in part by a grant from the Dr. Wallace C. and Clara A. Abbott Memorial Fund of the University of Chicago.

(fig. 1), although those treated with naphthaleneacetic acid attained slightly greater total development than the others (fig. 1*D*). The combination of the three and indoleacetic acid alone resulted in fruits of essentially the same sizes and remained green and turgid longest. The parthenocarpic fruits were firm and bright green in color for a period of 8–10 weeks, when they began to dry and shrivel. They attained as great a total length as fruits resulting from pollination but

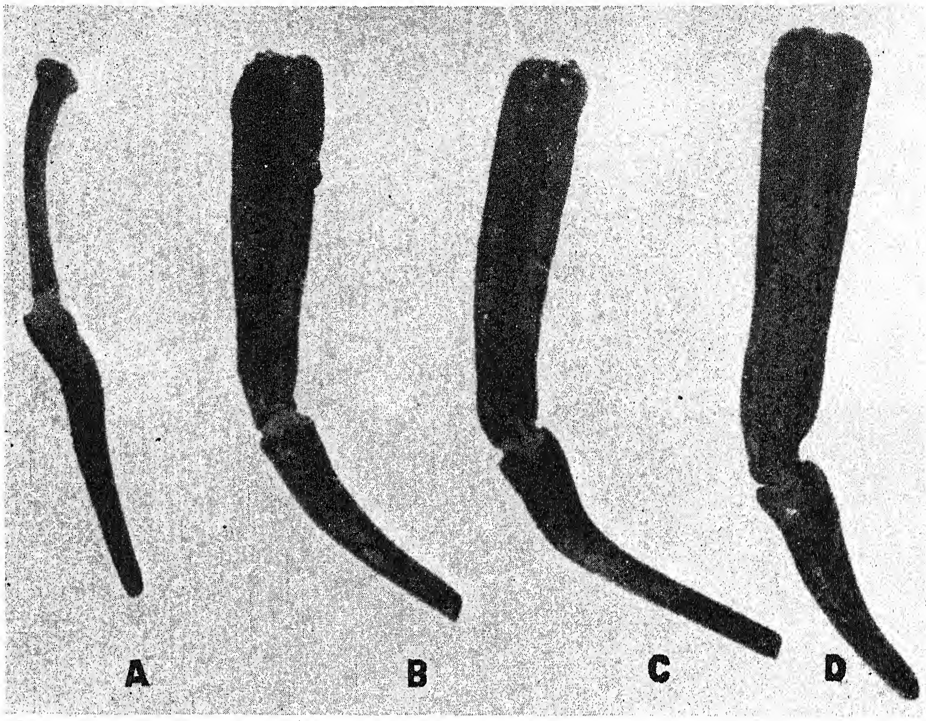

FIG. 1.—*A*, control, treated with lanolin alone, showing practically no change in size except at top end; *B*, 624 hours after treatment with combination of the three growth substances; *C*, 1152 hours after treatment with indoleacetic acid; *D*, 768 hours after treatment with naphthaleneacetic acid.

similar appearance (fig. 1*B*, *C*). A few of the treated ovaries showed initial responses to all the substances by starting to enlarge, and then growth ceased and they became dark and died. The majority of those treated responded as illustrated in figure 1*B*–*D*, and some at least remained green and turgid for more than 9 weeks. About fifteen of the plants were transferred to the garden in early July, transplanting from the pots being carried out without disturbing the roots. It was on these that the treated ovaries were always smaller in diameter. None of them developed embryos or endosperm.

Histological details

The photomicrographs are at two magnifications. All transverse sections of entire ovaries are at a magnification of about ×12, while those of individual ovules, both longitudinal and transverse, are magnified approximately ×60, or five times as much, in order to show more cellular detail.

The relative size and stage of development of ovaries and ovules attained at the time of treatment are shown in figure 2*A*. At this time the megagametophytes are mostly in or near the second four-nucleate stage, and mitotic figures are common in the carpel walls and in the integuments and nucellus of the ovules (fig. 2*A*, *B*). The inner integument and the nucellus have attained nearly full size, but the outer integument has not yet reached quite half the development it would show at the time of fertilization, which normally occurs about 100–110 hours following pollination in *L. regale*. The outer integument develops considerably during this period, ultimately covering the inner integument completely at the micropylar end before the pollen tube enters.

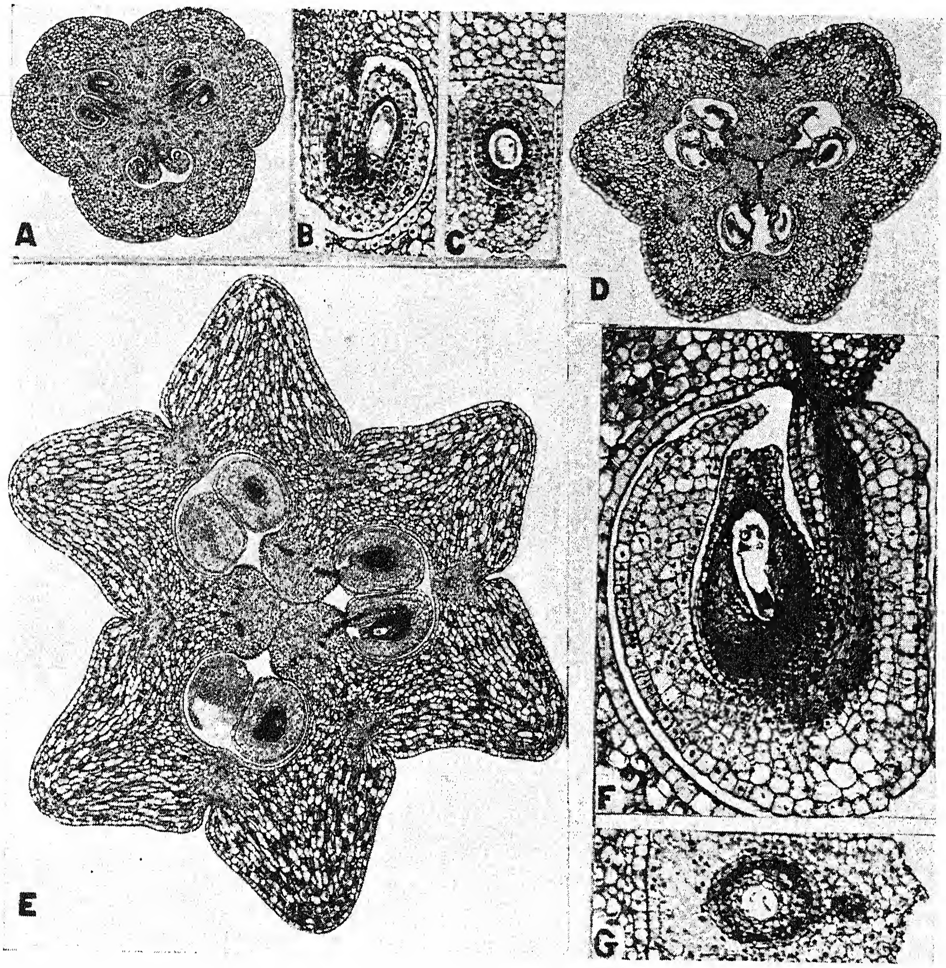

FIG. 2.—*A*, transection of control ovary at time of flower opening; *B*, ovule in longisection from same section as *A*, showing cellular detail; *C*, longisection of ovary at same stage as *A* and *B*, showing transectional view of an ovule; *D*, transection of ovary treated with lanolin alone, 552 hours after treatment; *E* transection of ovary 336 hours after treatment with naphthoxyacetic acid; *F*, ovule in longisection from *E*; *G*, transection of ovule from same ovary as *F*.

A transection of an ovary to which lanolin alone was applied, collected and fixed 552 hours after treatment (fig. 2*D*), shows evidences of enlargement in the carpel walls. Some of the growth is doubtless the result of increase in number of cells, but the greater part is a result of cell enlargement. The ovules, even though the outer integuments increased in volume, are now much shrunken and necrotic. When collected, this particular ovary was pale yellow in color and somewhat shrunken.

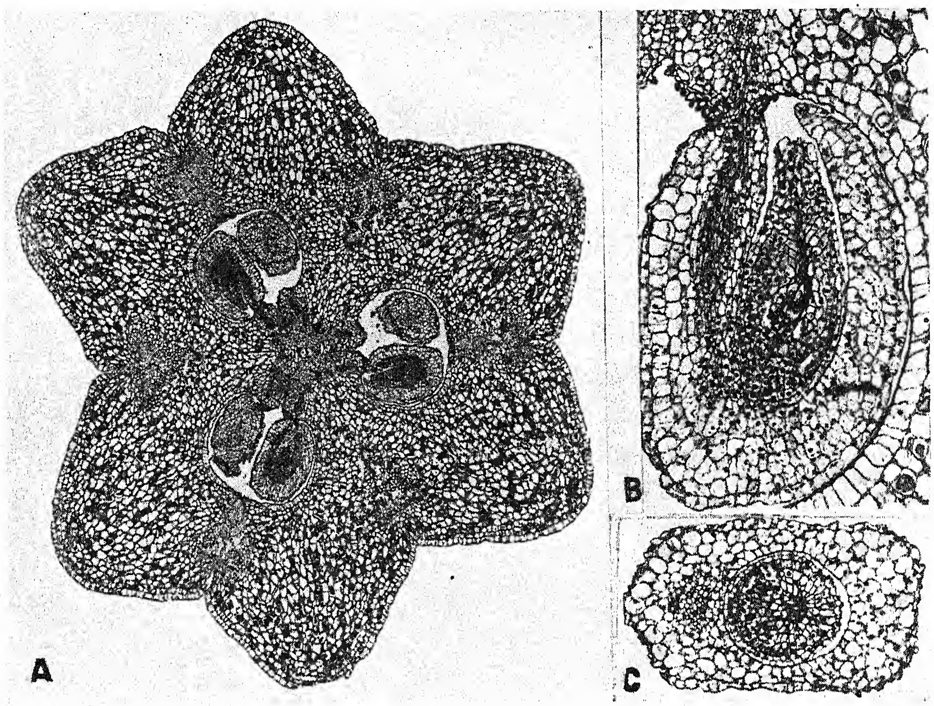

FIG. 3.—*A*, transection of ovary 480 hours after treatment with naphthoxyacetic acid; *B*, ovule in longisection from same section as *A*; *C*, transection of ovules from same ovary as fig. 3*A*.

The ovaries treated with the growth substances, either separately or in combination, behaved in much the same way in nearly all instances. At 336 hours after treatment with naphthoxyacetic acid the carpel walls and the ovules had increased in overall size about twofold or more (fig. 2*E*). The cells of integuments were still turgid, but the nucellus showed signs of disintegration (fig. 2*F*, *G*), and the cells composing the megagametophyte were partially collapsed and shrunken.

At 480 hours after treatment with naphthoxyacetic acid (fig. 3*A*) there was little further change in the appearance of carpel walls or in the integuments (fig. 3*B*, *C*). The megagametophyte, however, had now disintegrated completely and was observable only as a line more or less at the center of the nucellus.

Ovaries treated with a combination of the three growth substances developed at about the same rate as those to which the substances were applied separately. At 624 hours following treatment with the combination (fig. 4*A*) there was greater total development in carpel walls and in

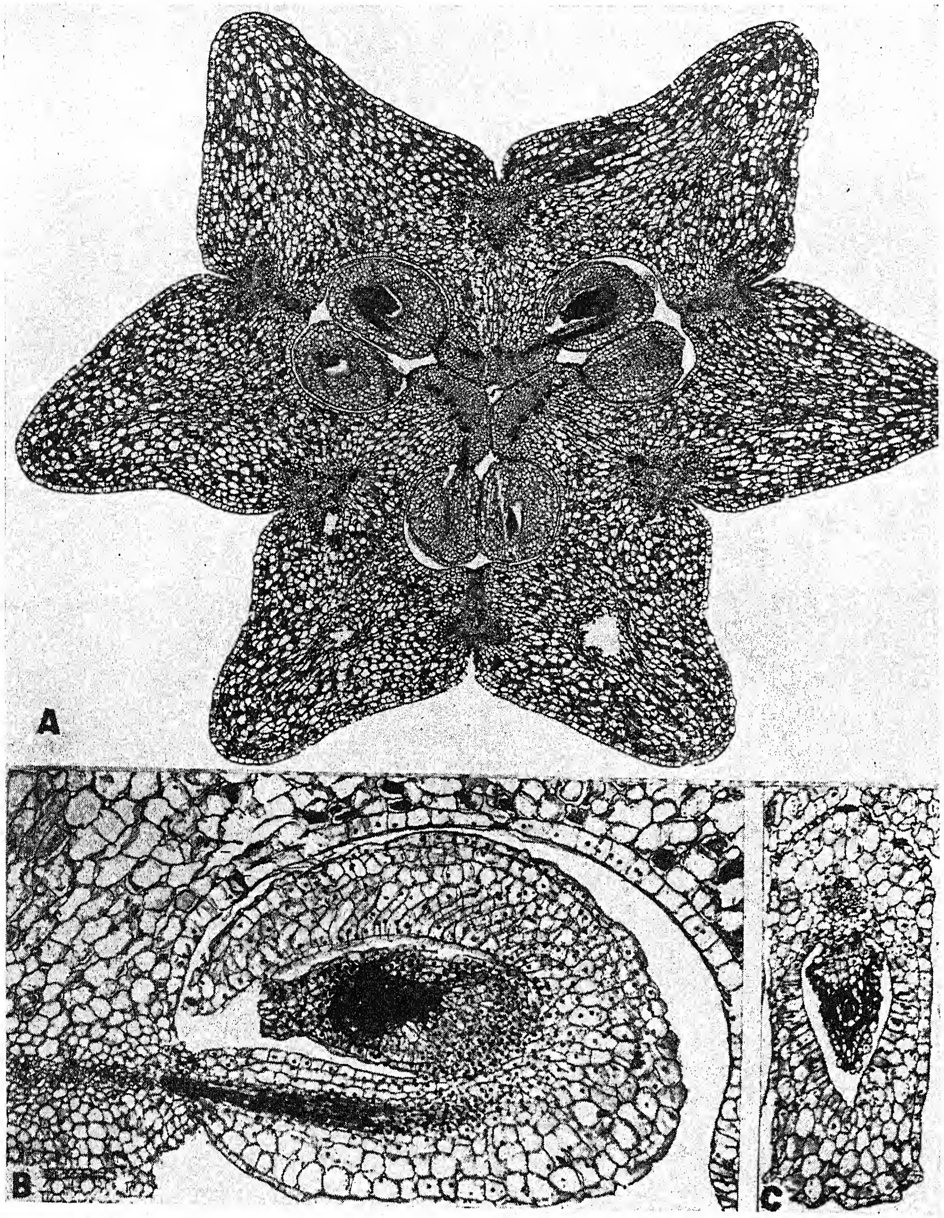

FIG. 4.—*A*, transection of ovary 624 hours after treatment with combination of the three growth substances; *B*, ovule in longisection from upper right of fig. 4*A*; *C*, transection of ovule from same ovary as fig. 4*A*.

the outer integument of ovules, but there were increasing evidences of degeneration in the inner integument and nucellus of the ovules (fig. 4*B*, *C*). Areas were also present in the carpel walls in which dissolution of cells had occurred, resulting in cavities which run parallel with the carpel walls.

A transection of an ovary collected 760 hours after treatment with naphthaleneacetic acid (fig. 5) gave further evidences of cellular effects in the inner portion of the carpel walls. The cavities resulting from cell disintegration were larger than those in figure 4*A*, and a band of collapsed cells was evident running almost entirely around the section. The overall size of ovules was greater, but the increase in volume was due entirely to growth of the outer integument. No cell divisions were apparent, and the increase in size was mainly the result of cell enlargement (fig. 6*A*, *B*). The inner integument and the nucellus were now undergoing disintegration.

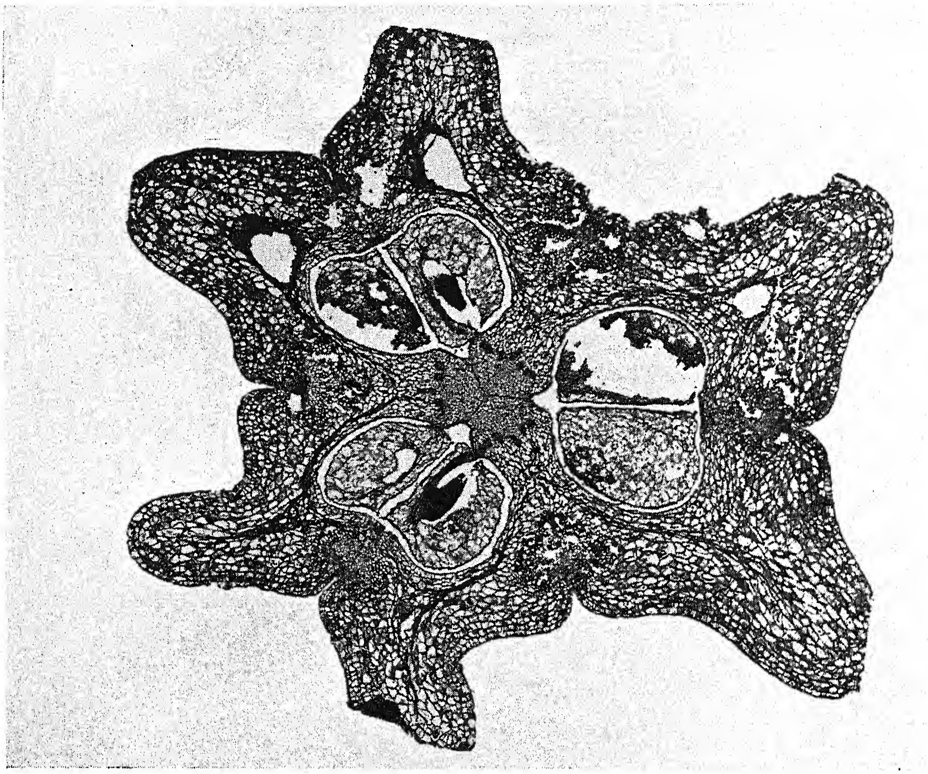

FIG. 5.—Transection of ovary 760 hours after treatment with naphthaleneacetic acid

The longest time interval following treatment at which material was collected and sectioned was 795 hours (33 days). A transection of such an ovary, treated with indoleacetic acid, showed well-developed carpel walls and large plump ovules (fig. 7). While the cavities in the carpel walls were somewhat larger than those in figure 5, there was less shrinkage and disorganization of cells in the inner walls of the carpels. But the

most striking development had taken place in the outer integument (fig. 8*A*, *B*). The marked increase in size had resulted from both cell divisions and cell enlargement, chiefly the latter. Indications of recent divisions were evident in several places in figure 8*A*, but the average volume of individual cells was much greater than in ovules at flowering time. Cells of the inner integument and nucellus were nearly all dead and much shrunken (fig. 8*B*).

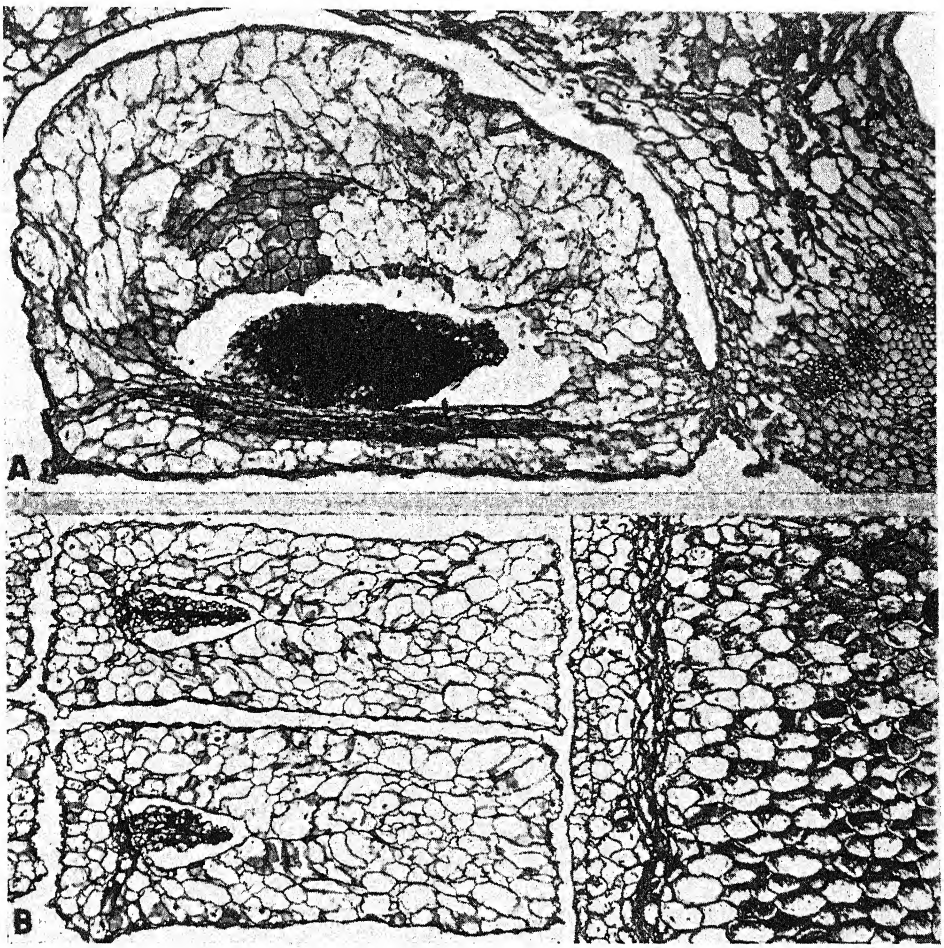

FIG. 6.—*A*, ovule in longisection from same ovary as fig. 5; *B*, transection of ovules from same ovary as fig. 5.

The general responses shown by the ovaries of *L. regale* to the three growth-promoting substances separately and to their combination were strikingly similar. For a time they resembled closely in external appearance those which developed as a result of pollination, but approximately 400 to 500 hours after treatment no further increase in diameter took place, and they remained more slender than those in which fertilization had occurred. They were similar in color

and felt as firm to the touch for 600 to 700 hours following treatment, after which time they usually became a paler green and began to show a slight pithiness. Both loss of color and pithiness increased slowly, until final death of the entire ovary occurred at 8–10 weeks following treatment.

The development of vascular bundles in the parthenocarpic fruits and those developed following pollination showed little difference. There was noticeable difference, however, in the appearance of the inner carpel wall tissues of parthenocarpic fruits as compared with those developed following pollination. In all the parthenocarpic fruits examined there was evident collapse of cells in the inner region of the carpel walls, followed by death and dissolution of at least some cells as early as 600 hours after treatment. While this effect appeared more strongly marked in some instances (following application of naphthaleneacetic acid, fig. 5) than in others, it nevertheless occurred in all the ovaries studied after a time lapse of approximately 600 hours following application of the growth substance.

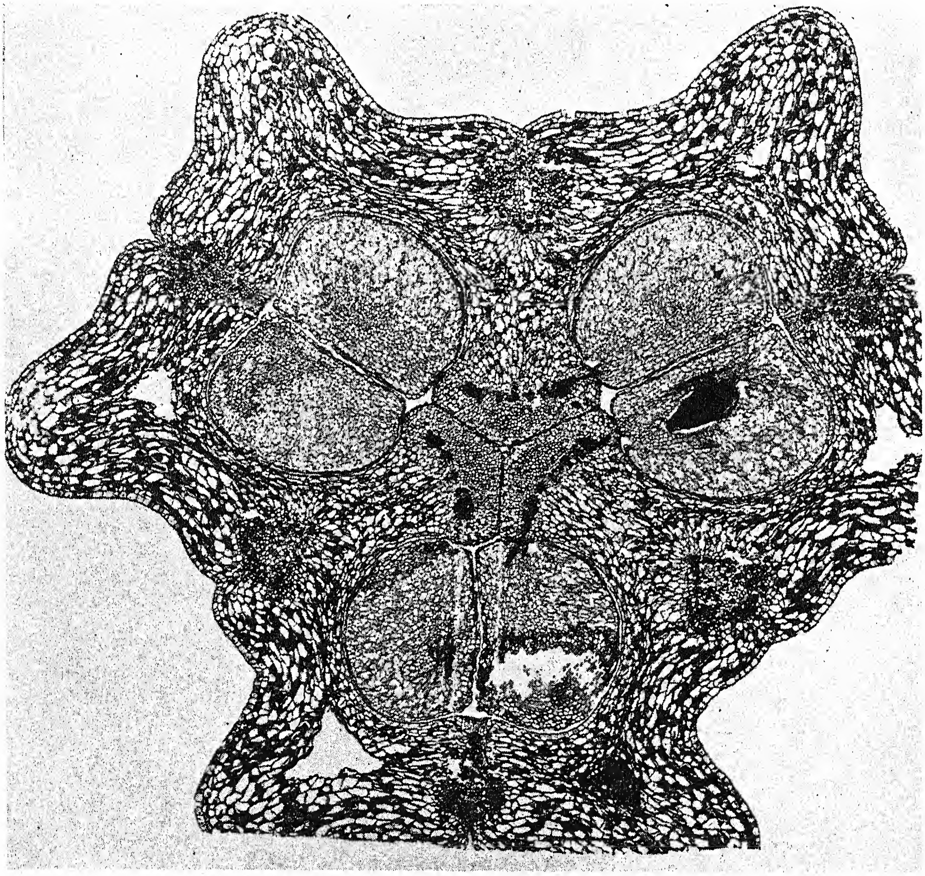

FIG. 7.—Transection of ovary 795 hours after treatment with indoleacetic acid

All the ovules of the parthenocarpic

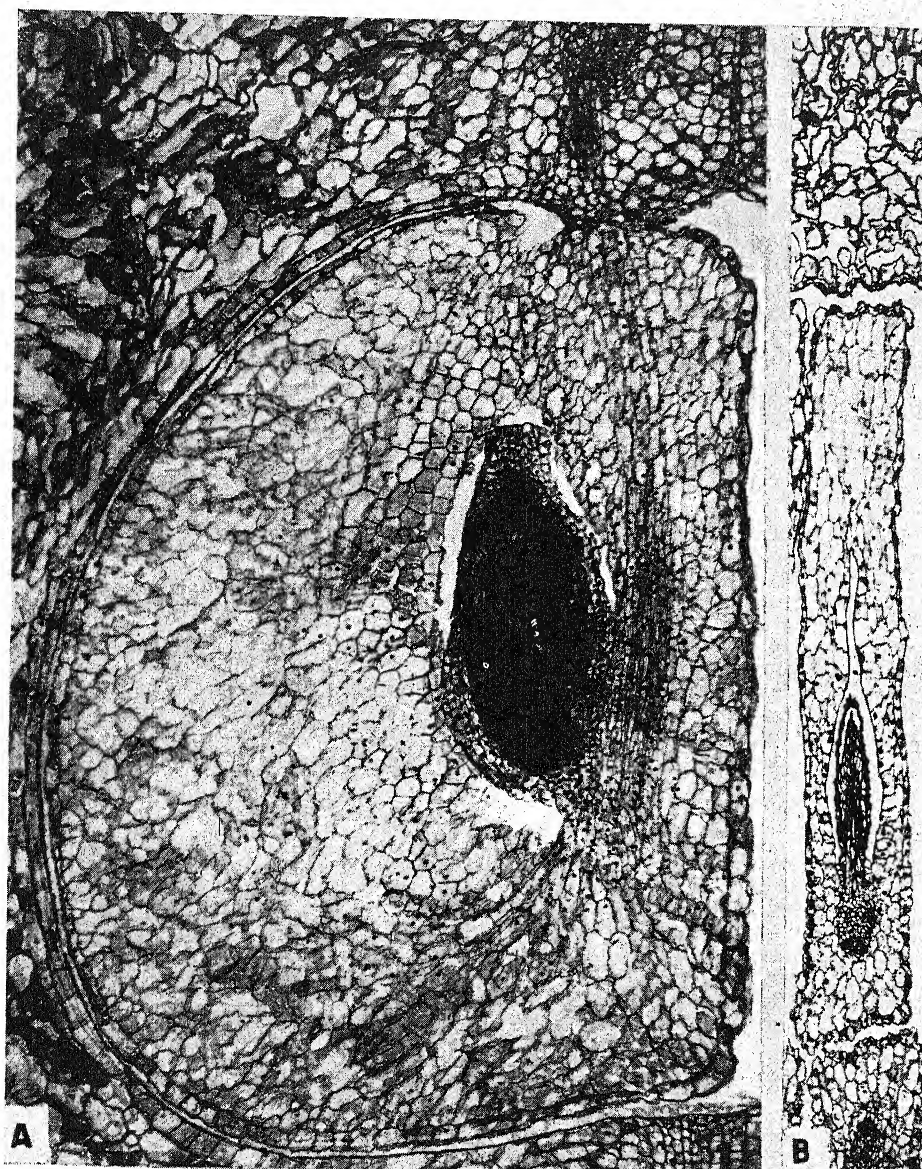

FIG. 8.—*A*, ovule in longisection from middle right of fig. 7; *B*, transection of ovule from same ovary as fig. 7.

fruits showed marked growth in the outer integument. The greater part of this growth was a result of cell enlargement rather than of cell multiplication, for once the outer integument had developed sufficiently to cover completely the micropylar end of the ovule, few additional mitoses occurred and subsequent growth was owing largely to increase in cell volume.

The most striking effects of the substances were the varied responses in specific regions and tissues of the ovaries. Few cell divisions occurred in the carpel walls subsequent to treatment. The conspicuous increase in size of ovaries resulted from enlargement of cells composing the carpels and outer integument of ovules. The shrinkage and collapse of cells in certain regions of the carpel walls, in the inner integument, the nucellus, and the mature megagametophyte occurred relatively early during the development of the parthenocarpic fruits. On the contrary, the cells of the outer integument were stimulated to increase in number to a limited extent, but chiefly they increased many times in volume. It is probable that other species of *Lilium* would show different degrees and types of responses to these same growth substances, as has been observed by WONG (4) for various species and varieties of cucurbits.

UNIVERSITY OF CHICAGO

LITERATURE CITED

1. GARDNER, F. E., and KRAUS, E. J., Histological comparison of fruits developing parthenocarpically and following pollination. BOT. GAZ. 99: 355–376. 1937.
2. GUSTAFSON, F. G., Inducement of fruit development by growth-promoting chemicals. Proc. Nat. Acad. Sci. 22:628–636. 1936.
3. ———, Parthenocarpy: natural and artificial. Bot. Rev. 8:599–654. 1942.
4. WONG, C. Y., Chemically induced parthenocarpy in certain horticultural plants, with special reference to the watermelon. BOT. GAZ. 103:64–86. 1941.

VEGETATIVE PROPAGATION OF TARAXACUM KOK-SAGHYZ WITH THE AID OF GROWTH SUBSTANCES

P. C. MARTH[1] AND C. L. HAMNER[2]

Introduction

Seedlings of *Taraxacum kok-saghyz* grown in the United States from imported seed vary considerably, both in plant character and in rubber content of the individual plants. Published data on vegetative propagation of kok-saghyz indicate that Russian investigators have had some success with piece-root cuttings. Plants produced by this method developed quickly under field conditions.

This report is based on greenhouse experiments conducted during December, January, February, and March, 1942–1943, at the Bureau of Plant Industry Station, Beltsville, on methods of handling piece-root cuttings of kok-saghyz and their rooting response to applications of synthetic growth substances.

Material and methods

Cutting material was obtained from separate lots of plants to determine the influence of age and size of root. One lot of approximately 3000 rather large, uniform roots, 7 months old, 10–15 inches long and $\frac{3}{4}$ inches in crown diameter, was obtained from a planting at St. Paul, Minnesota. In contrast to this material, cuttings were made of relatively young plants (4 months old) grown at Parma, Idaho, having roots 6–9 inches in length and with approximately $\frac{1}{4}$–$\frac{1}{2}$-inch crowns. In addition, 3000 plants 7 months old were obtained from a planting at Klamath Falls, Oregon. These roots were long (10–15 inches), straight, with few branches, and rather comparable in size with the plants from St. Paul. Two shipments of approximately 1000 plants each were received from Berkeley and from Davis, California. The former were coarse and much branched but fairly uniform, while the latter were variable in size, owing in part to irregular germination of the seed, which took place sporadically over a long period.

Four synthetic growth substances known to induce plant responses were applied in aqueous solution and by the talc-dust method. These compounds were β-indolebutyric acid, α-naphthaleneacetic acid, α-naphthalene acetamide, and β-naphthoxyacetic acid. A mixture made up of equal portions of these four compounds was also used. Each compound and the mixture were applied to the cuttings in a range of concentrations of 10, 50, and 100 p.p.m. with the solution method, and 50, 200, and 1000 p.p.m. in the talc-dust treatment.

Experimentation

Methods of propagation

Cutting length.—In the early stages of building up a clonal population, it is desirable to use the minimum length of propagating pieces that will succeed well and produce the maximum numbers of new plants. Uniform unbranched roots, approximately $\frac{3}{4}$ inch in diameter below the crown, that were obtained from the

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St. Paul planting were made into cuttings $\frac{1}{2}$, $\frac{3}{4}$, 1, and $1\frac{1}{2}$ inches long to determine the effect of cutting length on performance. One hundred cuttings of each size were set upright in sand in a propagating case operating at 70°–75° F. Periodic examination was made of a number of cuttings of each size group at intervals of 3–4 days, and at the end of 18 days all lots were removed for examination.

It was evident during the test period that the cuttings $\frac{1}{2}$ inch long were slower in callusing and producing new leaves than longer cuttings of $\frac{3}{4}$, 1, and $1\frac{1}{2}$ inches, and at the end of 18 days 70 per cent of the former had started to decay at the base. The $\frac{3}{4}$-inch pieces appeared to have made satisfactory callus growth at both ends, but this size was somewhat difficult to form into bundles, and the cuttings (twenty per bundle) tended to slip out. The size finally selected as a standard was the 1-inch length. A small vibrating motor-type saw was found useful in securing uniform lengths (fig. 1).

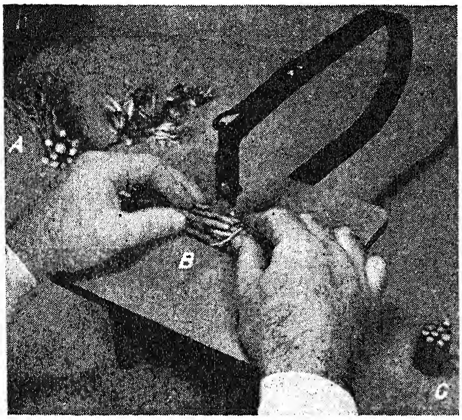

FIG. 1.—Method of making root cuttings of kok-saghyz: *A*, trimmed and bunched roots with tops removed; *B*, small vibrating saw; *C*, bundle of cuttings ready to be treated.

ORIENTATION.—Orientation with regard to the morphological top is of great importance in the successful rooting of most plants. To determine whether orientation is important with roots of kok-saghyz, separate lots of freshly made cuttings (St. Paul material) were set in sand vertically with the proximal end up; horizontally in a trench $\frac{1}{2}$ inch deep; horizontally on the surface of the rooting medium and covered with $\frac{3}{4}$ inch of moist sphagnum; and in an inverted position. The temperature of the cutting bench fluctuated from 70° to 80° F. during the 21-day test period. A minimum of 100 cuttings was used in each treatment, except with the inverted lot, which included 300 cuttings.

All the cuttings set with the proximal end up had developed leafy rosettes, and 43 per cent of them had developed some new root growth at the end of 21 days. In contrast with these results, out of 300 cuttings that were inverted, only four had developed leaves above the level of the media by the end of 21 days. The four cuttings that were the exception developed leafy growth on both ends. The remainder of the inverted lot (296) had all developed etiolated leaves below the surface and were very poorly rooted.

The humidity and temperature of the greenhouse or other imposed conditions apparently were very favorable for development of root rot, since all the cuttings laid horizontally and covered with $\frac{1}{4}$ inch of sand had decayed by the end of 21 days. In contrast, the cuttings placed horizontally on the surface of the sand and covered with moist sphagnum were in excellent condition at the end of the same period. Shoot and root growth, however, was much less extensive than with similar cuttings placed in sand in a vertical position with the proximal end up.

DEPTH OF SETTING.—Top growth was delayed and the incidence of decay was

greater with cuttings covered by the sand or soil rooting media. Three lots (100 in each) of 1-inch cuttings from the roots from St. Paul were set vertically in sand in a propagating case (70°–80° F.) at three depths of insertion: (*a*) top of cutting $\frac{1}{4}$ inch above media level; (*b*) top at media level; and (*c*) top $\frac{1}{4}$ inch below media level. By the end of 10 days all cuttings in the two lots with tops above or at media level had developed top callus upon which green leaves were visible. Both of these lots were maintained in the cutting bench without loss over a period of 18 days. On the other hand, the cuttings set $\frac{1}{4}$ inch below media level were just starting to callus at the end of 10 days, and 20 per cent had started to decay at the base at the end of 18 days. Likewise, in a separate experiment with cuttings held in sand in controlled temperature storage (40° ± 1° F.), the exposed cuttings developed greater callus than did those that were buried. At this temperature, however, cuttings buried under 1 inch of sand remained in good condition without loss from rot organisms over a 30-day storage period (March 16 to April 15).

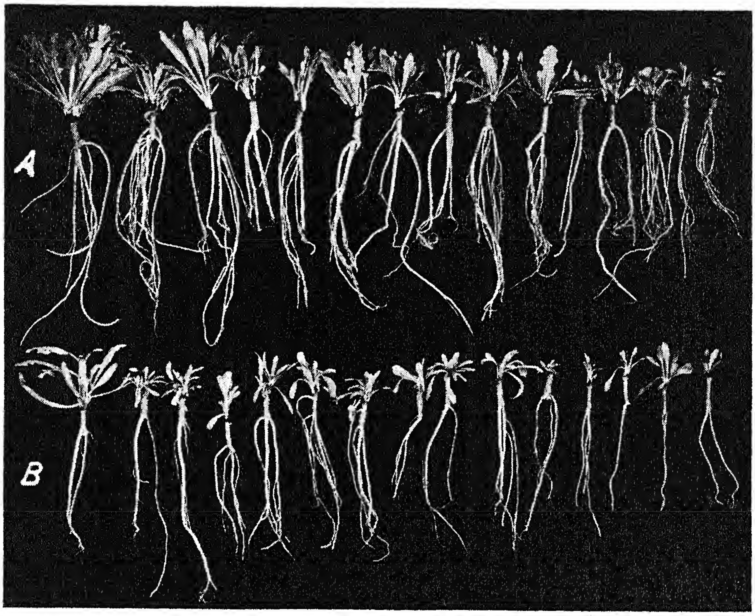

FIG. 2.—Plants from root cuttings placed in sandy soil in greenhouse on February 20, 1943; removed and photographed June 23. *A*, treated with 50 p.p.m. indolebutyric acid (16 hours); *B*, untreated controls.

CUTTING BENCH AND ROOTING MEDIA.—Under the greenhouse conditions employed in this study, inclosed cases were not necessary. Satisfactory rooting was obtained with open-type benches in a greenhouse at 60°–65° F.

Both a light sandy soil and a fine grade of washed bank sand were used as rooting media. Fifty cuttings of the Parma, Idaho, material were set in the sand on February 20 and left undisturbed for 30 days, while a similar lot was set in the sandy soil. By the end of the 30-day period the leaves on the cuttings in sand had begun to yellow, and approximately

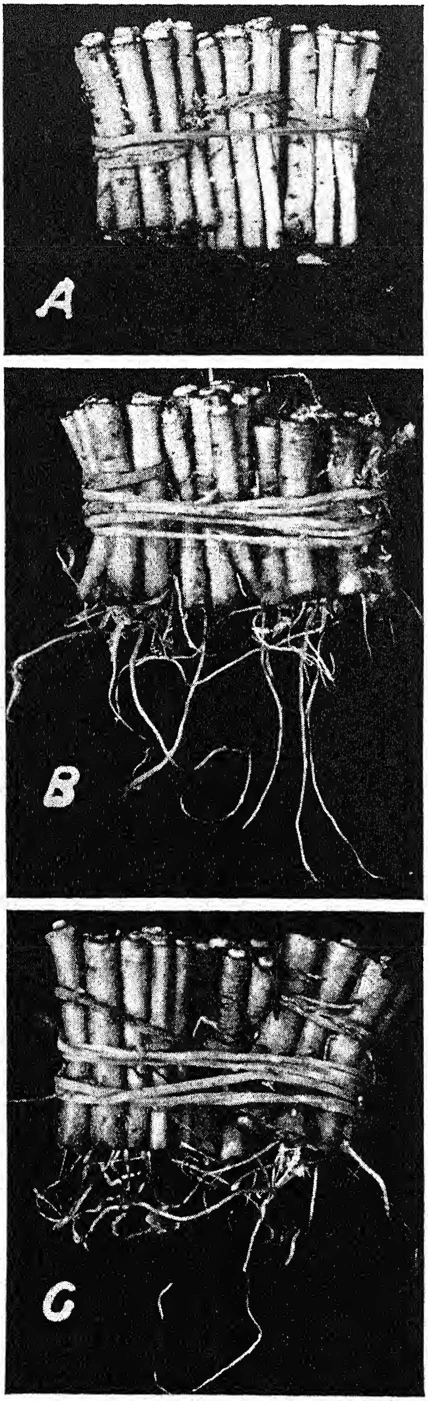

FIG. 3.—Root cuttings held in storage at 40° F.: *A*, control; *B*, solution-treated with 100 p.p.m. indolebutyric acid (16 hours); *C*, with 50 p.p.m.

60 per cent had made sufficient new root growth so that they survived potting into composted soil. Of the original fifty cuttings set in soil on February 20, forty-eight were living on June 23. A number of the resulting plants of this lot are shown in figure 2*B* as well as a comparable lot (fig. 2*A*) treated with growth substance (indolebutyric acid, 50 p.p.m.).

The wide variation in size found among these plants, the largest of which weighed 23.6 gm. while the smallest weighed but 1.8 gm. (fresh weight), was apparently due to differences in diameter size of the original cutting and to its relative position in the root from which it was made.

TEMPERATURE OF ROOTING MEDIA.—It was evident early in the work that rooting was affected by variation in temperature. Propagating cases with thermostatically controlled bottom heat were therefore used. The temperature ranges selected for trial were 60°–65°, 70°–75°, and 80°–85° F. Fifty cuttings (Klamath Falls, Oregon, plants) which received 50 p.p.m. indolebutyric acid (16-hour soaking) and fifty untreated cuttings were set in sand media maintained at each of the three temperatures on January 5.

Observations on these cuttings at periodic intervals indicated that the rate of callus production was noticeably faster at the higher temperature (80°–85° F.). At the end of 10 days all the cuttings at this temperature, both treated and untreated, had produced callus and green leaves over the entire exposed upper ends. Likewise, most of the cuttings in the 70°–75° frame were noticeably green at the upper cut surface. The cuttings set at 60°–65°, in contrast, were still almost completely dormant with but a very narrow ring of callus present on a few of them.

Final counts, when all lots were removed after 21 days in the cutting bench,

indicate that early callusing and shoot production are not reliable evidence of good rooting performance. The cuttings at the lower temperature had rooted in a much higher percentage, both in treated and untreated lots, and there was also no loss from decay. The percentage of cuttings with new roots and of dead cuttings at each of the three temperatures was as follows: Treated at 60°–65° F.: 76 per cent rooted, 0 dead; control 42 per cent rooted, 0 dead. Treated at 70°–75° F.: 74 per cent rooted, 22 per cent dead; control 32 per cent rooted, 16 per cent dead. Treated at 80°–85° F.: 8 per cent rooted, 86 per cent dead; control 18 per cent rooted, 72 per cent dead.

Treatment with 50 p.p.m. indolebutyric acid was apparently injurious at the higher temperatures (80°–85°), both in rooting and loss of cutting, while the same treatment applied to cuttings rooted at 60°–65° caused a highly significant increase in rooting without loss. Further evidence that growth substances are effective in inducing root production at even lower temperature was found in comparable treated (50 p.p.m. indolebutyric acid) and untreated cuttings stored in moist sphagnum and held in a controlled-temperature storage room at 40° F. for 30 days. During the storage period no new roots developed on the untreated cuttings, whereas 85 per cent of the treated ones had developed new roots, many of which were 3–4 inches in length (fig. 3).

Effectiveness of growth substances on rooting of cuttings

The use of growth substances as an aid in propagation of horticultural plants by stem cuttings is now common practice (2). Lindner (1) obtained a stimulating effect on rooting of horse-radish root pieces. Stuart and Marth (3), however, working with scion roots of twenty-six apple varieties, found no beneficial effects from use of growth substances.

TABLE 1

Effectiveness of growth substances on rooting performance and survival of plants from kok-saghyz root cuttings*

Compound†	Concentration (p.p.m.): Sixteen-hour soaking treatment	Concentration (p.p.m.): Talc-dust treatment	Percentage: Rooted cuttings after 18 days (Dec. 29–Jan. 16)	Percentage: Original cuttings which developed into plants, June 30
Ibc	100	....	97	27
	50	....	100	87
	10	....	70	70
	...	1000	60	60
	...	200	50	50
	...	50	47	43
Nac	100	....	100	10
	50	....	100	77
	10	....	63	47
	...	1000	60	53
	...	200	60	57
	...	50	53	43
Nad	100	....	87	53
	50	....	90	70
	10	....	73	60
	...	1000	53	53
	...	200	47	40
	...	50	47	43
Nxy	100	....	57	20
	50	....	73	73
	10	....	60	60
	...	1000	67	53
	...	200	57	57
	...	50	63	60
Mixture	100	....	93	47
	50	....	93	67
	10	....	67	53
	...	1000	60	57
	...	200	63	60
	...	50	43	43
Controls	16-hours in water	....	40	40
	....	Talc	37	33
	Untreated	....	30	27

* Thirty cuttings per treatment.

† Ibc, indolebutyric acid; Nac, naphthaleneacetic acid; Nad, naphthalene acetamide; Nxy, β-naphthoxyacetic acid; mixture, equal parts of the four growth substances.

The data in table I were obtained with cuttings of unbranched roots grown at Parma, Idaho. The thirty 1-inch cut-

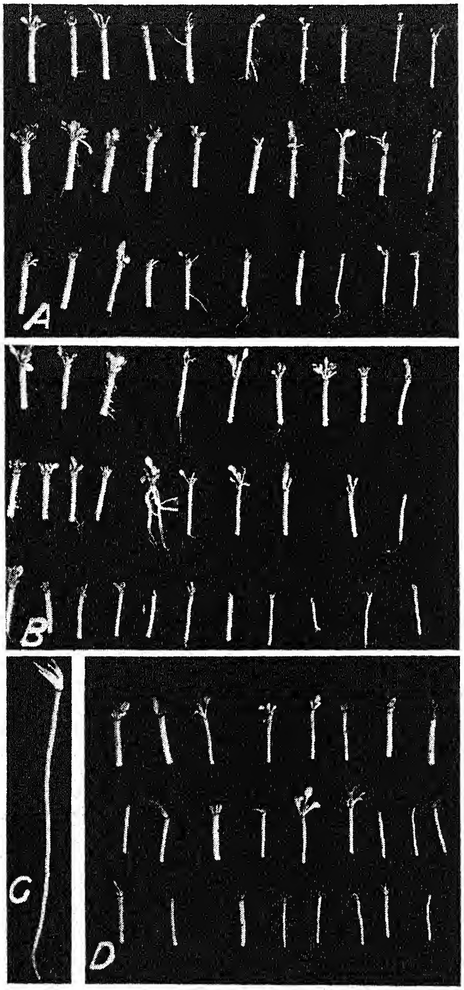

FIG. 4.—Root cuttings not receiving growth-substance treatment: *A*, water control (soaked 16 hours); *B*, untreated control (set directly into rooting medium); *C*, typical plant from which cuttings were made; *D*, talc dust only, control. Cuttings set in sand December 29, 1942; photographed 18 days later.

tings included in each treatment were selected at random, so that each lot consisted of an equal number (ten) from the upper (proximal), middle, and lower portion of the plants. The upper cuttings were approximately $\frac{1}{2}$–$\frac{5}{8}$ inch in diameter, while the lower cuttings were not less than $\frac{3}{16}$ inch. The cuttings were set in sand (68°–75° F.) on December 29, 1942, and removed after 18 days (January 16), at which time record was made of the rooted cuttings in each lot. These cuttings were then potted in soil and held in a cool (50°–65° F.) greenhouse. By April 26 a majority of the resulting plants had developed leaf rosettes 3–4 inches long, at which time they were field-planted. Counts were again made on June 30 of the living plants in each rooting treatment (table I).

Comparison of the effectiveness on rooting of the individual growth substances and the mixture employed can best be made with the 16-hour soaking treatments, rather than with the talc-dust method. As shown in table I, a marked increase over the controls was obtained from each growth substance applied in talc at the highest concentration used, 1000 p.p.m., and a large percentage of the rooted cuttings subsequently developed into plants. However, 100 per cent rooting was not approached in any of the dust treatments, nor was there any evidence of injury. It is possible, therefore, that the optimum concentration of growth substances in talc was not used in the particular range selected.

Although increased rooting over the control lots (fig. 4) was obtained from each of the growth substances and the mixture when applied in solution, the response to each compound was somewhat different.

INDOLEBUTYRIC ACID.—Following treatment with this growth substance, the plants tended to produce long fine roots, many of which arose from the new callus at the base of the cutting. Judging from observations on cuttings stored in

moist sphagnum, this compound induced more rapid rooting than the other growth substances. At a concentration of 50 p.p.m. (soaking treatment), 100 per cent rooting was obtained, and 87 per cent of these cuttings subsequently developed (table 1). At the stronger concentration of 100 p.p.m. the cuttings were injured (fig. 5). This is reflected in the very low number of plants (27 per cent) derived, even though the actual rooting percentage (97 per cent) was high at this concentration.

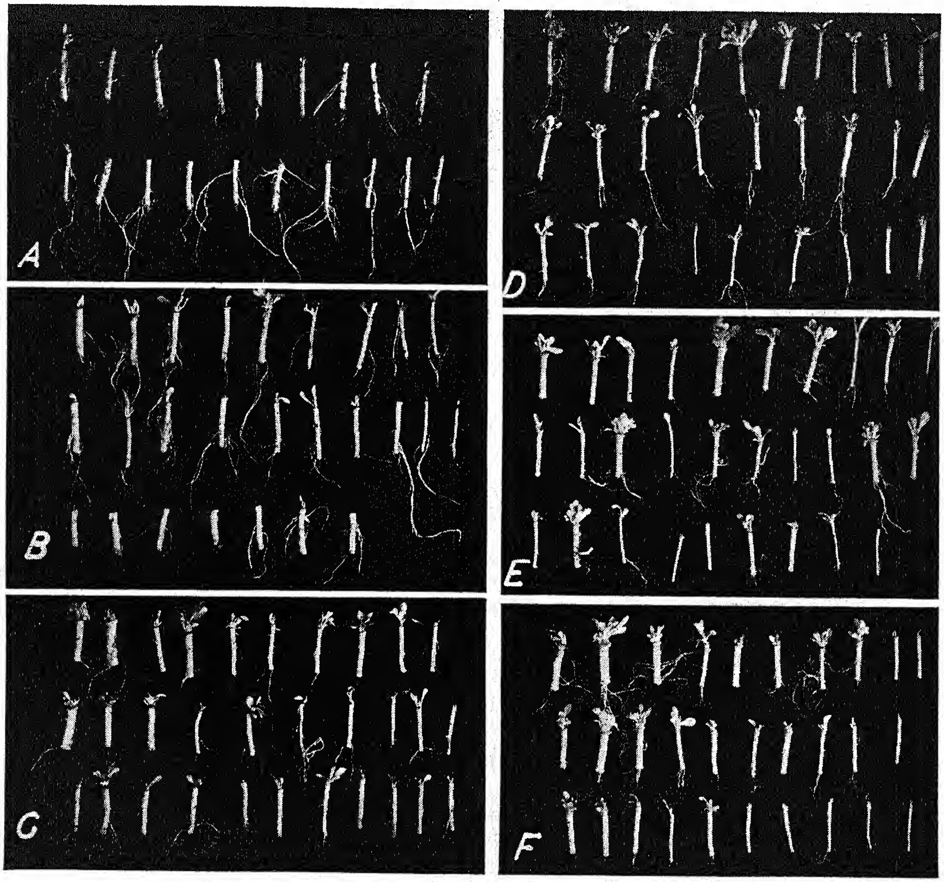

Fig. 5.—Root cuttings treated with indolebutyric acid: *A, B, C,* solution treatment at 100, 50, and 10 p.p.m., respectively; *D, E, F,* growth-substance–talc-dust treatments at 1000, 200, and 50 p.p.m. Cuttings set in sand December 29, 1942; photographed 18 days later.

Naphthaleneacetic acid.—This compound proved a very potent root-stimulating agent, inducing many short thickened lateral roots on the treated cuttings (fig. 6). It also was much more toxic at the higher concentration (100 p.p.m.) than the other compounds, and although all cuttings in this treatment had rooted strongly, 63 per cent showed injury when removed from the rooting media. The injury is further indicated by the low number of plants (10 per cent) resulting. The optimum concentration ap-

pears to be around 50 p.p.m. with the 16-hour soaking treatment. As indicated in table 1, all cuttings in this treatment were rooted after 18 days, and 77 per cent produced plants.

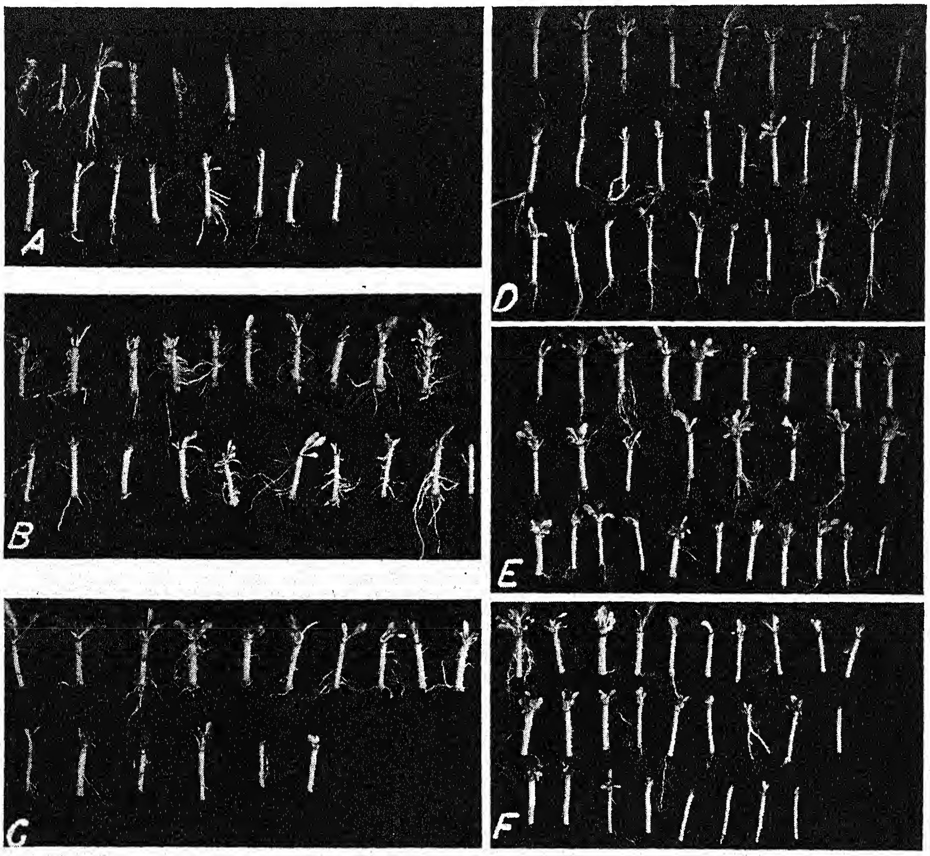

FIG. 6.—Root cuttings treated with naphthaleneacetic acid: *A*, *B*, *C*, solution treatment at 100, 50, and 10 p.p.m., respectively; *D*, *E*, *F*, growth-substance–talc-dust treatments at 1000, 200, and 50 p.p.m. Cuttings set in sand December 29, 1942; photographed 18 days later.

NAPHTHALENE ACETAMIDE.—This compound caused a marked increase in root production in comparison with the controls at each of the concentrations used—100, 50, and 10 p.p.m. (fig. 7). The increase, however, was less than with indolebutyric or naphthaleneacetic acid. Since some injury was caused at 100 p.p.m., it is assumed that the maximum concentration suitable for this type of material was approached. As shown in table 1, 50 p.p.m. with this compound gave the maximum rooting and ultimate plant yield. The roots developed on treated cuttings were thickened but slightly longer than those resulting from naphthaleneacetic-acid treatment.

β-NAPHTHOXYACETIC ACID.—Following treatment with this compound, cuttings had few, but very long fine roots (fig. 8). On the basis of root production alone, β-naphthoxyacetic acid would be rated much less satisfactory than the

other three compounds. However, with the exception of the 100 p.p.m. treatment, where injury occurred, the sparsely rooted cuttings resulting from treatment yielded fairly high percentages of plants (table 1).

MIXTURE.—The maximum rooting obtained with the use of a growth-substance mixture (at 50 p.p.m.) was not so great as with either indolebutyric or naphthaleneacetic acid alone. At 100 p.p.m., however, the mixture was noticeably less toxic than any of the compounds applied singly (fig. 9). Perhaps in its favor also is the fact that the type of roots developed on the cuttings treated with the mixture tended to be somewhat intermediate between the long fine roots characteristic of the cuttings treated with indolebutyric acid and the short coarse roots of those treated with naphthaleneacetic acid.

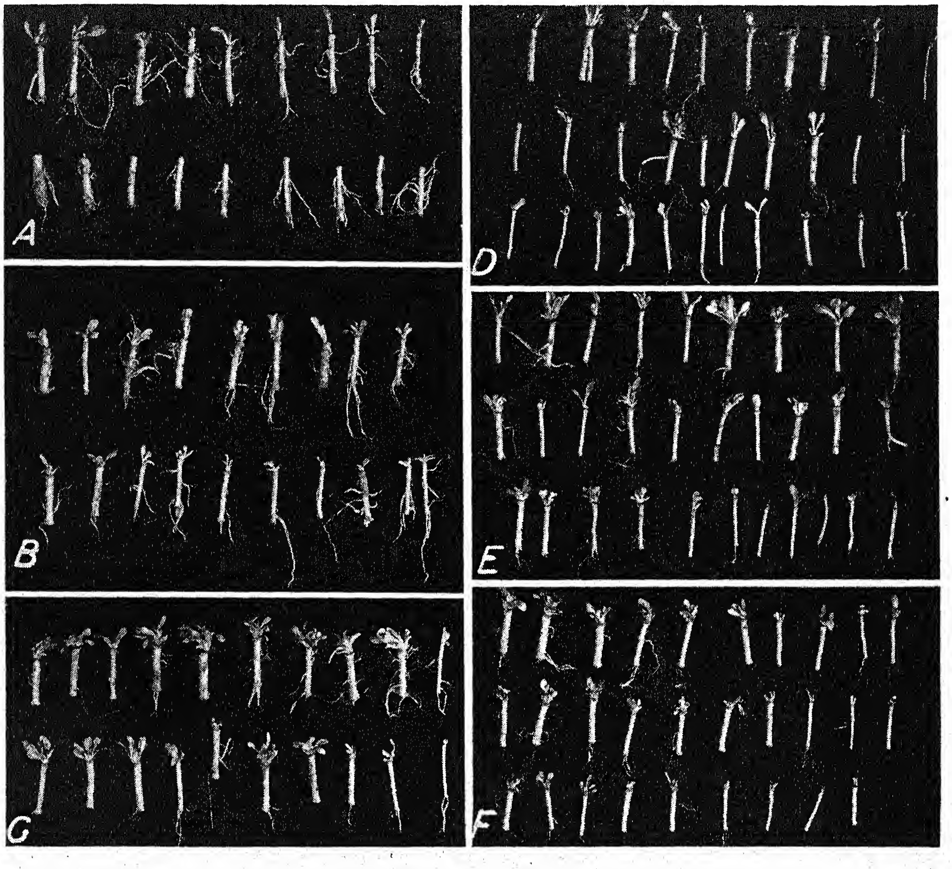

FIG. 7.—Root cuttings treated with naphthalene acetamide: *A, B, C,* solution treatment at 100, 50, and 10 p.p.m., respectively; *D, E, F,* growth-substance–talc-dust treatments at 1000, 200, and 50 p.p.m. Cuttings set in sand December 29, 1942; photographed 18 days later.

GROWTH-SUBSTANCE TREATMENT OF INTACT PLANTS

After the roots have been cut into small (1-inch) pieces, there is some difficulty in telling which is the morphologi-

cal top. It is relatively easy for the bundles to become reversed in the course of handling and many are likely to be planted upside down. There would be some advantage, therefore, in having a satisfactory growth-substance treatment for the intact plants before the cuttings were made. The cuttings could then be made up from the treated roots just prior to insertion in the rooting medium, with less likelihood of reversing them.

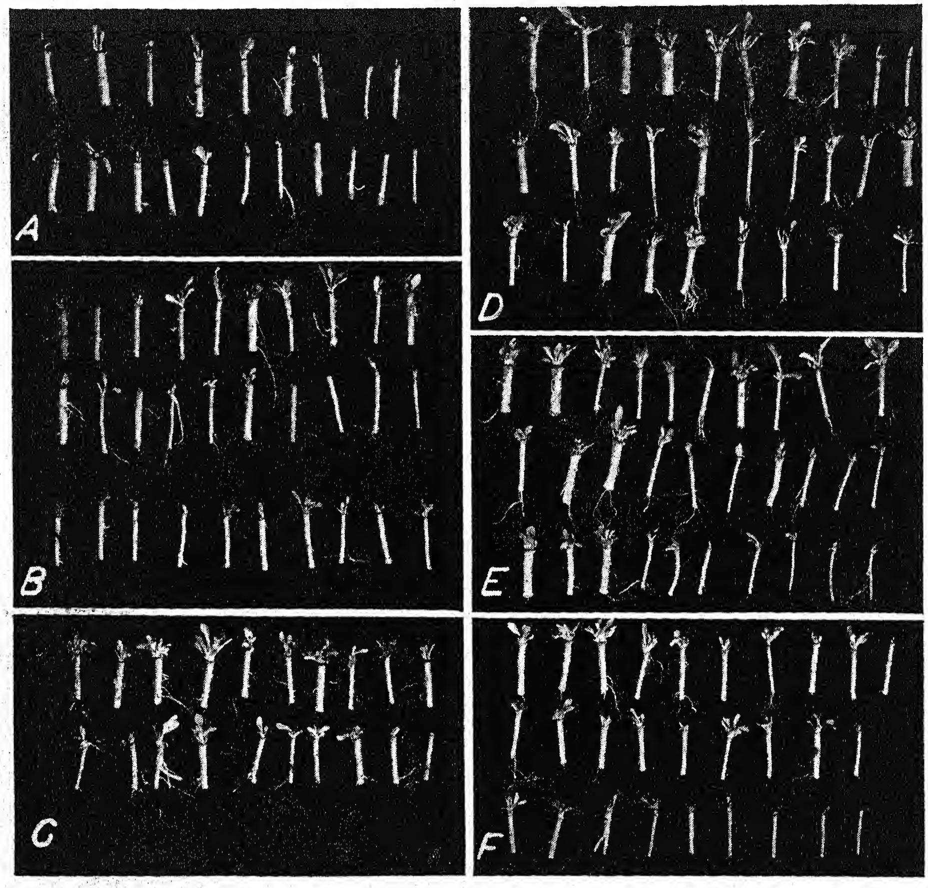

FIG. 8.—Root cuttings treated with β-naphthoxyacetic acid: *A*, *B*, *C*, solution treatment at 100, 50, and 10 p.p.m., respectively; *D*, *E*, *F*, growth-substance–talc-dust treatments at 1000, 200, and 50 p.p.m. Cutting set in sand December 29, 1942; photographed 18 days later.

Uniform lots of ten entire plants grown at Klamath Falls, Oregon, were soaked for 16, 32, and 64 hours in aqueous solutions of 0, 10, 50, and 100 p.p.m. of each of the four growth substances and the mixture, while similar lots were soaked for 2, 4, and 8 hours in stronger solutions of 0, 200, 400, 800, 1000, and 1400 p.p.m. of the same compounds. For comparison, an additional control lot was planted without soaking. The attempt was thus made to compare a treatment consisting of a long soaking period at dilute concentrations with a shorter soaking period at higher concentrations. Upon removal

from the solutions, the plants of each treatment were cut into uniform pieces 1 inch long, thereby yielding 75–80 cuttings in each lot. The rooting medium was a sandy loam soil, and the last treated lot was set on February 2, 1943. Final record on all was obtained at the end of 30 days (March 4).

Under the conditions employed, increased rooting did not result from treatment of intact plants with growth substances before the cuttings were made. Although the results were complicated by considerable damage in all lots from rot organisms, greater loss of cuttings was found in the treated than in the untreated lots, with the exception of the 16-hour treatments at 10 and 50 p.p.m. and of the 2- and 4-hour treatments at 200 and 400 p.p.m. The percentage of rooted cuttings in each of these lots treated with growth substance was no greater than that from either the untreated plants or those soaked in water only.

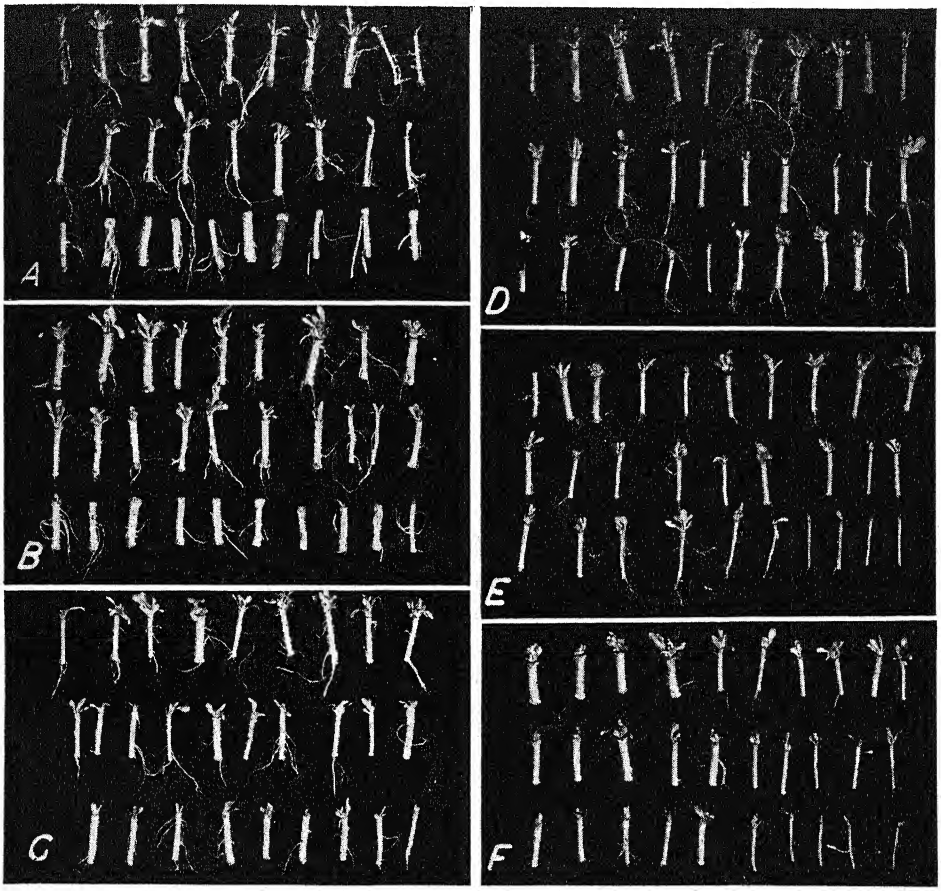

FIG. 9.—Root cuttings treated with mixture of growth substances: *A*, *B*, *C*, solution treatment at 100, 50, and 10 p.p.m., respectively; *D*, *E*, *F*, growth-substance–talc-dust treatments at 1000, 200, and 50 p.p.m. Cuttings set in sand December 29, 1942; photographed 18 days later.

Prolonged soaking for 32 and 64 hours, either in water only or with growth sub-

stances added, appeared to be harmful. Likewise strong concentrations of 1000 and 1400 p.p.m. with 8 hours of treatment also appeared definitely injurious. All the cuttings in each of these lots died as a result of rot or other causes by the end of the 30-day period.

FIG. 10.—Upper: plants developed from root cuttings. Lower: close-up of some of same plants to show the profuse flower development obtained.

FIELD DEVELOPMENT OF PLANTS FROM CUTTINGS.—The majority of the plants from cuttings that were rooted in the greenhouse, potted in soil, and held in a cool (40°–55° F.) greenhouse and then planted in the field on April 20, produced an abundance of flowers. Many of the larger plants had rosettes 5–10 inches in diameter and had produced 25–40 flowers per plant by June 20 (fig. 10). During warm days, however, all the plants from cuttings were severely wilted by noon but returned to a turgid condition in the

evening. A number of plants were therefore lifted for root examination.

In contrast to the relatively large amount of top growth produced by these plants, root growth was much restricted. Even the larger (10-inch) ones had developed but two to three sizeable roots per plant, the longest of which was but 2 inches.

Apparently the top-root ratio of kok-saghyz plants produced from root cuttings may be markedly altered by cultural conditions imposed upon the developing plants (fig. 2). Root cuttings set in soil in a cool greenhouse (60°–65° F.) on February 20 had developed much greater root than top growth by June 23.

Summary

1. Root cuttings of more than 9000 plants of *Taraxacum kok-saghyz* were used in experiments during December, January, February, and March, 1942–1943, to determine the response of cuttings to variations in propagation methods and to applications of β-indolebutyric, naphthaleneacetic, and naphthoxyacetic acid, and napthalene acetamide. Each compound was used in concentrations of 10, 50, and 100 p.p.m. in solution treatments and at 50, 200, and 1000 p.p.m. in talc dust.

2. The most desirable length of cutting was 1 inch, with a minimum diameter of $\frac{3}{8}$ inch. Cuttings $\frac{1}{2}$ inch in length were slow in callusing and rooting, while cuttings $\frac{3}{4}$ inch long were somewhat difficult to handle and form into bundles.

3. The roots had strong polarity, and most pronounced rooting responses were obtained with cuttings set with the proximal portion up. Inverted cuttings failed to develop normally, while those laid horizontally were retarded in growth of tops and roots.

4. Under greenhouse conditions, cuttings with tops at or slightly above the level of the rooting medium developed new top and root growth more quickly than cuttings buried $\frac{1}{4}$ inch below the surface.

5. Both sand and a light sandy soil were used as a rooting medium with equal success. The cuttings set in soil developed into sizeable plants over a 4-month period, while with similar lots set in sand it was necessary to remove them to soil at the end of 30 days.

6. Although root cuttings developed callus and top growth more quickly at 80°–85° F., these cuttings were more susceptible to rot and the percentage rooted was lower than with similar cuttings maintained at 60°–65° during the rooting period (21 days).

7. Treatment with growth substances by the solution methods resulted in an increase in the percentage of rooted cuttings in comparison with untreated controls. The optimum concentration for best results with the compounds used was at 50 p.p.m. with a 16-hour solution treatment. Each of the four growth substances and the mixture resulted in injury at 100 p.p.m.

8. The growth-substance–talc-dust treatments induced a higher percentage of rooted cuttings than was found in the untreated lots. At the highest concentration (1000 p.p.m.), however, rooting was less than with the same compounds applied at 50 p.p.m. in solution.

9. At the optimum concentration of growth substance for rooting (50 p.p.m.) indolebutyric and naphthaleneacetic acids produced a higher percentage of rooting than either naphthalene acetamide, β-naphthoxyacetic acid, or the mixture used.

10. The roots produced by cuttings

following treatment with indolebutyric and β-naphthoxyacetic acids tended to be long and fine, while those which developed with napthaleneacetic acid and naphthalene acetamide tended to be short and much thickened.

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LITERATURE CITED

1. LINDNER, R. C., Effects of indoleacetic and naphthylacetic acids on development of buds and roots in horse-radish. BOT. GAZ. 100:500–527. 1939.
2. MITCHELL, J. W., and RICE, RUBY R., Plant-growth regulators. Misc. Publ. 495. U.S. Dept. Agr. 1942.
3. STUART, N. W., and MARTH, P. C., Experiments with growth substances on rooting of scion roots of apple. Unpublished data. U.S. Dept. Agr.

EFFECTS OF HIGH TEMPERATURE ON METAPHASE PAIRING IN LILIUM LONGIFLORUM

S. L. EMSWELLER AND PHILIP BRIERLEY[1]

The regularity of nuclear and cell divisions in plants has been considerably affected by many agents, such as X-rays, ultraviolet light, various chemicals, diseases, and abnormal temperatures. Both low and high temperatures, as well as a sudden change from one to the other, have been shown to be particularly effective.

The writers have previously briefly discussed (4) some of the meiotic irregularities that arose in *Lilium longiflorum* as the result of a sudden change from low to high temperature. In the present paper these abnormalities are presented in some detail.

Two horticultural forms of *L. longiflorum* were employed. One was the Creole variety that is grown in Louisiana and along the Gulf Coast and the other was the Japanese type known as Giganteum. The latter should not be confused with the species *L. giganteum* Wallich. The Creole variety is believed to be a clone because of its uniformity, its self-incompatibility (2), and certain cytological peculiarities not observed in any of the many other *longiflorum* varieties so far examined (5). The variety Giganteum is actually a mixture of various types, as is shown by its variability in many flower characteristics, its stem color, and the incompatibility relations between individual plants.

Twelve Creole and nine Giganteum plants, all with buds in which some of the anthers were at the leptotene and early pachytene stages, were selected. At this time the buds on the Creoles ranged 13–32 mm. in length, and those on the Giganteums 8–36 mm. One week before the treatments were given, the temperature in the greenhouse where the plants had been growing was lowered to 10°–13° C. at night and 13°–16° C. during the day. On March 19 the plants were divided into two lots. Six Creoles and five Giganteums were moved to a warmer greenhouse, where the temperatures were 13°–16° C. at night and 16°–19° C. during the day. The remaining six Creoles and four Giganteums were placed in a chamber where the temperature was raised in about 3 minutes to 45°–46° C. A thermocouple placed inside one of the buds showed that the temperature was maintained at 45.5° ± .5° C. during the entire exposure of 30 minutes. High humidity was maintained by introducing steam into the chamber. Following the treatment the plants were placed in the warm greenhouse with the untreated ones. Anthers were collected at intervals from both treated and untreated plants, starting when many of the pollen mother cells were in first metaphase. The time required for such development varied to some extent, apparently being dependent on the size of the bud at the time it was exposed to the high temperature treatment. The anthers were fixed in 3:1 absolute alcohol-acetic acid, then transferred through several stages of alcohol to 80 per cent, where they were stored until slides were made. The ordinary

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aceto-carmine smear technique was used, and the photomicrographs were made with a Zeiss microscope and Miflex camera.

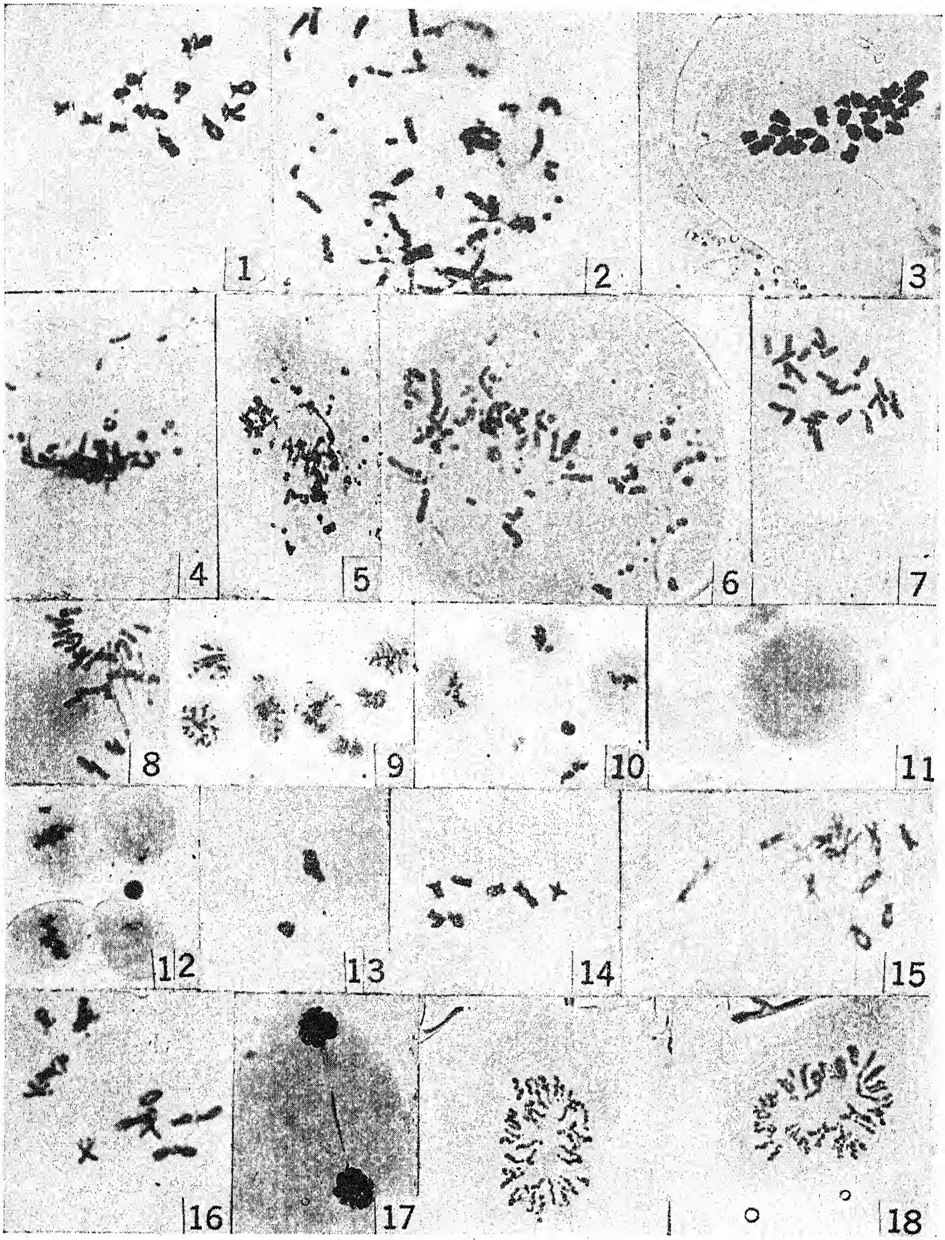
Some remarks regarding metaphase bivalent formation in the untreated Creole and Giganteum plants have already been presented (5). The more detailed data on chromosome pairing in the untreated plants are presented in table 1. It was possible to examine carefully only one flower each from three of the Creoles and two each from the remaining three plants. As shown in the table, univalents were found in eight of the nine buds investigated. In addition there was an unusual condition in which the chromosomes were broken into small fragments, in many instances so numerous that it was impossible to count them. The condition is referred to in this paper as "blown-up." Four cells of this type are shown in figures 2, 4, 5, 6. Figure 2 was collected from an untreated Creole plant on March 23, 91.5 hours after the others were treated; figures 4 and 6 are from treated plants collected March 25, 138.5 hours after treatment; figure 5, also treated, was collected March 27, 48 hours later. Figure 1 is a normal Creole pollen mother cell with 12 bivalents, shown for comparison with the blown-up cells. It was collected at the same time as figure 2. Some of the blown-up cells (figs. 4, 6) closely resemble irradiated ones, while others (fig. 5) are very similar to the condition found in sticky gene of maize (1).

TABLE 1

METAPHASE PAIRING IN TWO VARIETIES OF LILIUM LONGIFLORUM (UNTREATED)

Plant no.	Flower bud no.	No. cells observed	Cells showing chromosomal conditions indicated: $12_{II}+0_{I}$	$11_{II}+2_{I}$	$10_{II}+4_{I}$	Blown-up cells	Large p.m.c.
			Creole				
1	1	74	51	19	4	......	......
2	1	49	40	9	......	......	......
3	1	16	13	2	1	......	......
	2	142	93	16	......	33	......
4	1	137	116	8	......	13	......
	2	71	68	......	......	1	2
5	1	132	100	26	......	4	2
6	1	46	36	7	......	3	......
	2	67	56	7	......	3	......
Total	......	734	573	94	5	57	4
			Giganteum				
1	1	49	49	......	......	......	......
	2	50	50	......	......	......	......
	3	33	32	1	......	......	......
2	1	40	40	......	......	......	......
3	1	48	48	......	......	......	......
4	1	59	57	2	......	......	......
5	1	68	68	......	......	......	......
Total	......	347	344	3	......	......	......

This unusual fragmentation in blown-up cells is first seen at late prophase. The long chromosomes appear to be broken into many pieces, most of which have a tendency to be attached to the general mass by fine attenuated threads. These cells are always larger than the surrounding normal ones and are found scattered throughout the anther. At earlier stages of meiosis large cells have also been observed, but they do not show any indication of the fragmentation seen in the pollen mother cells at later satges. At first metaphase the blown-up cells usually show an apparent spindle, and occasionally a few abnormal bivalents may be seen among the mass of fragments. Some of the large fragments apparently move to both poles, since second metaphases have been observed in which some of the many chromosomes appeared to be undergoing normal mitotic splitting. Occasional microspores are seen that contain only a few chromosomes, and it is possible that some of these arose from blown-up cells At all stages of development these abnormal cells are larger than usual, as may be seen by comparing figures 1 and 2, photographed at the same magnification.



FIGS. 1–18.—*Lilium longiflorum:* Fig. 1, pollen mother cell of Creole variety with 12 bivalents. Fig. 2, "blown-up" pollen mother cell from untreated Creole plant. Fig. 3, large Creole pollen mother cell with 24 shortened chromosomes, resulting from suppression of heterotypic division (from same bud as fig. 2). Figs. 4–6, blown-up cells from treated Creoles. Fig. 7, pollen mother cell from bud 1 of treated Creole plant 3, showing complete asynapsis. Fig. 8, another pollen mother cell from same bud as fig. 7, showing 3 bivalents and 18 univalents. Fig. 9, five pollen mother cells from one anther of bud 1 of Creole plant 3, showing variable asynapsis. Fig. 10, three pollen mother cells from bud 2 of same plant as fig. 9, showing complete asynapsis. Fig. 11, enucleate pollen mother cell from bud 2 of Giganteum plant 1. Fig. 12, four pollen mother cells from same source as fig. 11, one with 12 bivalents, one with 11 plus 2 univalents, one with a heteromorphic bivalent, and one with a fragment. Figs. 13–16, pollen mother cells from same Giganteum plant as figs. 11 and 12, with 1 bivalent plus a fragment, 7, 14, and 13 bivalents, respectively. Fig. 17, chromatid bridge in pollen mother cell of treated Creole plant. Fig. 18, second metaphase in Creole, probably later stage of fig. 3. Figs. 1–4, 6, 13–17, ×600; figs. 5, 11, ×400; figs. 7, 8, 18, ×500; figs. 9, 10, 12, ×300.

The blown-up cells are closely associated with ones showing normal behavior, and their frequency varies from plant to plant and even in buds from the same plant. This variation (table 1) has been observed each year during the last four growing seasons. Sometimes anthers have been collected and slides prepared that did not contain a single blown-up cell; a week later slides made from a younger bud on the same plant have shown this condition. In the anthers of untreated Creoles there also occurs an occasional large pollen mother cell with 24 shortened chromosomes. One is shown in figure 3. This was from the same plant and collection that produced the blown-up cell shown in figure 2. Only four cells of this type were observed among the 734 recorded in table 1. They are always larger than normal, being about the same size as the blown-up cells. All four observed in the untreated plants were at the same stage of development as the one shown in figure 3. Such cells probably divide to form diploid dyads. Figure 18 shows the two nuclei of such a dyad, each with 24 chromosomes.

The first metaphase in Giganteum is very regular (table 1). Three buds on plant 1 and one each on the remaining four plants were examined. Only three in the 347 cells observed showed any divergence from complete pairing, and in these only 2 univalents per cell were found. Additional observations of meiosis in Giganteum during other seasons have never revealed any of the blown-up or large pollen mother cells found in the Creole variety.

The effects of the 30-minute exposure at 45°–46° C. on the Creole and Giganteum plants were not so severe as might have been expected. Some buds showed external injury, and a few were aborted. No buds collected for observations on meiosis showed any indication of injury as a result of the treatment. The effects on metaphase pairing are recorded in tables 2 and 3. In the Creole three buds were examined from plant 1, two each from plants 2, 3, and 5, and only one bud each from plants 4 and 6. As shown in table 2, all the irregularities occurring in the untreated Creoles were found in each of the treated plants. In addition, the degree of asynapsis was greatly increased in flower-bud number 1 of plant 3 and in bud number 2 of plant 5. A cell from bud 1 of plant 3 with complete asynapsis is shown in figure 7. Figure 8 shows another cell from the same source, with 3 bivalents and 18 univalents. Every possibility of chromosome association, from complete pairing to complete asynapsis, was found, although not in any one flower bud. In addition, a few first metaphase figures with fragments were seen. Some of these fragments were probably associated with the formation of dicentric chromatids. Figure 17 shows a cell from a treated Creole plant with a chromatid bridge that has broken at two points. Unfortunately the available anthers were rather limited in number, and not many late stages of meiosis were observed. Similar chromatid bridges have been seen in untreated Creole plants; no data are available as to whether the frequency is increased by the high temperature treatment.

Treatment on two buds on the same plant shows divergent effects. In figure 9 is shown a group of five highly asynaptic cells from Creole bud number 1 of plant 3 for comparison with the regular pairing (fig. 10) in the three cells from bud 2 of the same plant. There is also considerable difference in frequency of large pollen mother cells and blown-up cells in the two buds of plant 3 (table 2). The blown-up cells in bud 2 were found in groups of

TABLE 2

METAPHASE PAIRING IN TWO VARIETIES OF LILIUM LONGIFLORUM (HIGH TEMPERATURE TREATMENT)

PLANT NO.	FLOWER BUD NO.	NO. CELLS OBSERVED	Cells showing chromosomal conditions indicated														
			$12_{II}+0_{I}$	$11_{II}+2_{I}$	$10_{II}+4_{I}$	$9_{II}+6_{I}$	$8_{II}+8_{I}$	$7_{II}+10_{I}$	$6_{II}+12_{I}$	$5_{II}+14_{I}$	$4_{II}+16_{I}$	$3_{II}+18_{I}$	$2_{II}+20_{I}$	$1_{II}+22_{I}$	$0_{II}+24_{I}$	Blown-up cells	Large p.m.c.
			Creole														
1........	1	117	29	29	6	2	0	0	0	0	0	0	0	0	0	45	6
	2	17	11	4	0	0	0	0	0	0	0	0	0	0	0	2	0
	3	77	10	10 (1+f)	3	0	5	0	0	1	0	0	0	0	0	45	2
2........	1	88	54	4	3	0	0	0	0	0	0	0	0	0	0	26	1
	2	56	9 (1+f)	20	9	5 (1+f)	0	0	0	1	0	0	0	0	0	12	0
3........	1	59	0	0	0	1	0	0	0	6 (1+f)	8	9	13	9	10	3	0
	2	201	42	22	5	1	1	0	0	1	0	0	0	0	0	102	27
4........	1	71	52	9	0	0	0	0	0	0	0	0	0	0	0	10	0
5........	1	264	210	2	0	0	0	0	0	0	0	0	0	0	0	46	6
	2	62	0	3 (1+f) (1+3f)	1	1	2	5	6	0	0	0	0	5	4	35	0
6........	1	170	58	33	5	0	1	0	0	0	0	0	0	0	3	56	14
Total	......	1182	475	136	32	10	9	5	6	9	8	9	13	14	17	383	56
			Giganteum														
1........	1 2	82 (see | 0 table 3)	0	0	0	0	0	0	0	0	4	8	15	55	0	0
2........	1	39	39	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	2	36	36	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	3	39	37	2	0	0	0	0	0	0	0	0	0	0	0	0	0
....	1	52	0	0	1	1	3	4	4	12	13	8	3	3	0	0	0
....	1	(see | table 3)														
Total	......	248	112	2	1	1	3	4	4	12	13	12	11	18	55	0	0

three to five, but it is difficult to be certain of such relationships in a smear preparation.

The data in table 2 on the treated Giganteums show almost complete inhibition of chromosome pairing in flower-bud number 1 of plant 1 and greatly reduced pairing in the bud from plant 3. Plant 2 exhibited no effect in any of the three buds examined. No large pollen mother or blown-up cells were found in any of the treated Giganteum plants. The observations on bud 2 of plant 1 and on the one of plant 4 are presented in table 3. The differences in types of abnormalities (tables 2 and 3) between the two buds on the Giganteum number 1 are striking. All the cells from the first bud were diploid and showed a high frequency of asynapsis. The cells from the second bud were mostly aneuploids, and bivalent formation was very high. The two buds were probably at different stages of development when treated, with bud number 2 probably in a pre-meiotic condition. From bud number 2, 192 cells were examined critically and found to fall into thirty-nine types with regard to their chromosome content and behavior. These types included enucleate pollen mother cells and those with every number of bivalents, from 1 to 15. Some of these types also had one or more fragments; and one with 4 bivalents included a trivalent. The occurrences of types most frequently found were: thirty-six cells with 12 bivalents (2n), thirty-six with 13 bivalents (2n + 2), nineteen with 14 bivalents (2n + 4), and ten with 1 bivalent (2n − 22).

TABLE 3

METAPHASE PAIRING IN LILIUM LONGIFLORUM, VARIETY GIGANTEUM (HEAT TREATED)

Condition of pairing	No. of cells showing condition indicated: Flower bud no. 2, plant 1	No. of cells showing condition indicated: Flower bud no. 1, plant 4
Enucleate	4	1
f	2	....
1+0	10	1
2+0(1+f)	7	....
2+clumps	2	....
3+0	6	....
4+0	5	....
4+2 clumps	1	....
$4+1_{III}$	1	....
5+0	5	1
5+2 clumps	1	....
6+0	5	....
6+clump	1	....
7+0	5	....
7+clump+f	1	....
8+0	5	....
8 senescent clumps	1	....
9+0	6	1
9+6	1	....
10+0	2	1
11+0	7	1
11+1	2	1
11+2	2	....
11+3+ff	1	....
11+f	2	....
12+0	36	43
12+1	2	....
12+1+f	1	....
12+2	2	1
12+f	1	....
13+0	36	17
13+1	2	....
13+2	2	....
13+1+f	1	....
13+f	1	1
13+ff	1	....
14+0	19	....
14+f	1	1
15+0	2	....
Total	192	70

The pollen mother cells in the bud from plant 4 (table 3) did not exhibit

such a wide assortment of types as did those in the bud of plant 1. There was a much greater frequency of the normal type, with 12 bivalents, but there was also a high frequency of cells with 13 bivalents (2n + 2). Since there always existed the possibility of some chromosomes being lost because of the smear technique, the data are from only those cells about which there was no question regarding this point. Some of the cells from bud 2 of plant 1 and the one bud from plant 4 are shown in figures 11–16. In figures 11 and 12 the cells were not flattened, and the unbroken cell walls can be seen. Figure 11 is typical of the enucleate cells, and figure 12 shows a group of four cells, all different with respect to the quantity or behavior of their chromatin content. The topmost cell of the four has 11 bivalents and 2 univalents; the one below and to the right has only a small fragment; the cell below and to the left has 12 bivalents; and the lowermost cell has a heteromorphic bivalent. Figures 13 and 14 show two deficient types, the former with 1 bivalent and a fragment and the latter with 7 bivalents. Types with 14 and 13 bivalents, respectively, are shown in figures 15 and 16.

Discussion

The Creole and Giganteum varieties of *L. longiflorum* reacted very differently to the same heat treatment, although as a rule these varieties are highly cross-compatible (2). In general, the meiotic irregularities observed in both varieties exposed to 45°–46° C. for 30 minutes may be divided into three classes: (*a*) those concerned with rearrangements within individual chromosomes; (*b*) those in which partial to complete asynapsis was induced; and (*c*) those in which either the entire nucleus was eliminated or chromosomes were added to or subtracted from the normally expected number.

The first group includes the so-called blown-up cells and the fragments in other cells. The origin of the blown-up cells is obscure. Although pollen mother cells in an anther are closely associated in the same environment, only a few blown-up cells were formed. Nevertheless, since the high temperature did materially increase the frequency of blown-up cells, the aberration may to some extent be controlled by the environment. All these blown-up cells were larger than normal, the increase being already present in prophase cells, so that the breakdown must have occurred during the very early stages of meiosis or possibly during the last pre-meiotic mitosis. This entire problem needs further investigation. The same aberration has been found by STEWART (9) to occur naturally in pollen mother cells of *L. pumilum*. The affected cells in this species are also larger than normal and show the same high frequency of fragmentation as occurs in *L. longiflorum*. They also occur with very high frequency, whereas only a few were found in *L. longiflorum*. The *L. pumilum* plants in which the cells occurred were from a group of seedlings many of which did not reveal any blown-up cells. It will be interesting to see whether this peculiar behavior is found in other members of the genus *Lilium*.

The fragments found in cells not blown-up probably arise from crossing-over in inverted sectors as well as from non-homologous associations. Chromatid bridges and fragments were seen in cells from both treated and untreated plants.

The suppression of chromosome pairing was greatly increased by the heat treatment. In complete asynapsis the

chromosomes lay scattered in the cytoplasm. Such cells were comparable in size with those in which pairing was complete (figs. 1, 7). The large pollen mother cells with 24 shortened chromosomes, however, apparently had a different origin. These large cells were always found in anthers in which the other cells were almost all at first metaphase. They probably resulted from suppression of the first division, although it is difficult to understand how their volume was increased so rapidly.

The complete elimination of all chromatin, or the addition or subtraction of chromosomes, in two different heat-treated Giganteum plants probably occurred at the last pre-meiotic mitosis. Enucleate cells, and ones with but a fragment, can persist up to the first meiotic division. In some cells with but 5–6 bivalents the chromosomes were about to separate, indicating that such cells can at least initiate meiosis. Unfortunately later stages of meiosis were not available, since all anthers were collected as soon as first metaphase figures were found.

The effect of exposure to high temperature on nuclear and cell divisions in other species of plants has received considerable attention from other investigators. TAKAGI (10) found that a 3–3.5-hour exposure of *Lychnis sieboldii* to a temperature of 38°–39° C. produced three types of asynapsis. In the first type the heterotypic division was suppressed, the unpaired chromosomes split, and diploid dyads were formed. The second type differed from the first in that the 24 unpaired chromosomes were assorted at random, forming tetrads of two types with respect to chromosome number. In the third type each univalent split in prophase of the first division and no interkinetic nuclei were formed, the chromosomes lying scattered in the cell. SAX (7) subjected plants of *Rhoeo discolor* that had been grown for 2–3 days at 10° C. to a temperature of 36° C. for 1 day. This resulted in suppression of meiosis and the production of dyads which developed into diploid pollen grains. Some tetraploid pollen grains were also formed as a result of the failure of nuclear division following chromosome splitting. Later the same investigator (8) studied the effect of sudden temperature changes on meiosis in *Tradescantia paludosa*. The treatments were most effective when plants were transferred from 8° to 38° C. Such chromosomal aberrations as interchanges, inversions, fusions, and fragmentations were induced. Asynapsis, or suppression of chromosome segregation at meiosis, was also produced, resulting in diploid pollen grains. DERMEN (3) found that fragmentation, fusion, and chromatid bridges were induced in *Rhoeo discolor* by changing from either a low to a high temperature or the reverse. Tetraploid pollen mother cells were formed as a result of doubling the number of chromosomes in pre-meiotic tissue of the anthers. KAGAWA (6) studied the effect of abnormal temperature on the course of pollen formation in a genus hybrid, *Triticum compactum* × *Secale cereale*. The plants were first grown at 4° C. for 39 hours, then at 30° for 7 hours. The results were classed into: (*a*) nonreduction (asynapsis); (*b*) formation of restitution nuclei; (*c*) formation of a nucleus containing all the chromosomes of the pollen mother cell in one of the two cells formed by the first division, while in the other cell no chromatic content was observed. The third abnormality sometimes varied because of the failure of all chromosomes to be included in one of the two cells formed by the first division. As a result, one of the cells

would contain most of the chromosomes and the other only one or two. This is the same type of behavior that occurred in two of the Giganteum plants.

Summary

1. Plants of the Creole and Giganteum varieties of *Lilium longiflorum* with young flower buds were removed from a cool greenhouse (10°–16° C.) and exposed for 30 minutes to a temperature of 45°–46° C. They were then returned to a greenhouse with temperature of 13°–19° C.

2. As a result of this treatment, the frequency of all the irregularities normally found in Creole plants was greatly increased. These included (*a*) so-called "blown-up" cells, in which all or practically all the chromosomes were broken into numerous fragments; (*b*) formation of restitution nuclei; and (*c*) increase in the degree of asynapsis from partial to complete. In Giganteum, partial to complete asynapsis was induced, and in two buds on different plants enucleate pollen mother cells were found as well as others with only a fragment or with bivalents ranging in number from 1 to 15. The treatment evidently affected both the last pre-meiotic mitosis and the early stages of the meiotic cycle.

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LITERATURE CITED

1. Beadle, G. W., A gene for sticky chromosomes in *Zea mays*. Zeitschr. Indukt. Abstamm. Vererb. **63**:195–217. 1932.
2. Brierley, Philip, Emsweller, S. L., and Miller, J. C., Easter lily breeding: Compatibilities in *Lilium longiflorum* stocks. Proc. Amer. Soc. Hort. Sci. **34**:603–606. 1936.
3. Dermen, Haig, Cytological analysis of polyploidy induced by colchicine and by extremes of temperature. Jour. Hered. **29**:211–229. 1938.
4. Emsweller, S. L., and Brierley, P., Effect of high temperature on metaphase pairing of *Lilium longiflorum*, Giganteum and the Creole lily. Abstract, Proc. Amer. Soc. Hort. Sci. **35**: 104. 1937.
5. ———, and ———, Metaphase pairing in *Lilium longiflorum* "Giganteum" and the "Creole" lily. Abstract, Proc. Amer. Soc. Hort. Sci. **35**:824. 1937.
6. Kagawa, F., The effect of abnormal temperature on the course of pollen formation in a genus hybrid, *Triticum compactum*×*Secale cereale*. Jap. Jour. Bot. **10**:55–68. 1939.
7. Sax, K., The experimental production of polyploidy. Jour. Arnold Arbor. **17**:153–159. 1936.
8. ———, Effect of variations in temperature on nuclear and cell division in *Tradescantia*. Amer. Jour. Bot. **24**:218–225. 1937.
9. Stewart, R. N., Observations on spontaneous abnormalities in meiosis of *Lilium pumilum*. Unpublished.
10. Takagi, F., The influence of higher temperature on the reduction division of the pollen mother cells of *Lychnis sieboldii*. Sci. Rep. Tohoku Imp. Univ. **3**:461–466. 1928.

CELL GROWTH AND NUCLEIC ACIDS IN THE POLLEN OF RHOEO DISCOLOR

THEOPHILUS S. PAINTER

Introduction

The studies of CASPERSSON and coworkers, BRACHET, and others have shown that when there is rapid synthesis of complex substances such as proteins taking place in cells, as in the formation of ova, embryonic development, malignant or other types of growth, or the elaboration of complex secretory products (pancreas), considerable quantities of ribose nucleic acids are invariably present in the cytoplasm and usually heavy deposits of chromatin in the nuclei.[1] The presence of ribonucleic acid in the cytoplasm causes the latter to stain with basic dyes, and in older publications, especially on plant cytology, many have indicated the presence of basophilic materials in growing tissues. Not all basophily in the cytoplasm is due to the presence of ribonucleic acids, however, and the identification of the latter can be made either through ultraviolet absorption studies (5) or by the use of ribonuclease enzymes linked with appropriate methods of preservation and staining (2). The latter method has been employed in the present study.

In *Rhoeo* there are three periods of marked increase in the size of pollen mother cells and their derivatives by true growth (that is, the synthesis of new cytoplasmic and nuclear materials). The first of these includes the usual growth which the pollen mother cells undergo just prior to the meiotic divisions and represents about a fourfold increase in cell volume. The second period of growth comes as the microspores change into mature pollen grains and represents about a fifteenfold volume increase. The third period begins when pollen is placed on the stigma of the flower and includes the formation of the pollen tube.

Material and methods

Incidental to another experimental study of chromosomes, it was noted that in *Rhoeo* the cytoplasm becomes extremely basophilic in microspores as they undergo development into mature pollen. Tests showed that this basophily is removed when the microspores are first treated with ribonuclease, indicating that the basophilic material is made up of ribose nucleic acids.[2] For the study reported here, BRACHET'S technique (2, 3) was employed. Anthers in various stages of development were dissected from flower buds and preserved in Helly's fluid. Sections were either stained in Unna's methyl green-pyronin mixture or were treated first with ribonuclease (kindly furnished by Dr. Kunitz) in the following manner. Sections were brought into distilled water in which a small amount of crystalline ribonuclease was dissolved. Such slides were kept at a temperature of about 50° C. for 2–3 hours. Control slides were similarly treated except for the ab-

[1] The term ribose nucleic acid is used throughout this paper in the sense of polymerized ribose nucleotides and thus is not restricted to the four nucleotides carrying adenine, cytosine, guanine, and uracil, which are conventionally represented in most diagrams of ribose nucleic acid.

[2] See ALLEN, F. W., The biochemistry of the nucleic acids, purines, and pyrimidines. Ann. Rev Biochem. 10:221. 1941.

sence of the enzyme. After treatment and washing in distilled water, experimental and control slides were placed back to back, stained, differentiated, and dehydrated together, so that each pair of slides received just the same treatment. Anthers dissected in aceto-carmine give about the same color reactions found by the longer Helly-Unna procedure. A number of anthers were also fixed in Navashin's fluid, stained by Feulgen's method, and counterstained in light green.

In following the changes in the anthers during the development of pollen, and those which occur when pollen is placed on the stigma of the flower, interest centers primarily on the reaction of the cytoplasm of the pollen mother and other cells to the basic dye pyronin. When basophilic material is present it causes the cytoplasm to stain a bright red with pyronin, and since the basophilic material is removed by ribonuclease the intensity of the red color in the cytoplasm of untreated cells is taken as an indication of the amount of ribonucleic acid present at a given stage. In the drawings the various shades of red in the cytoplasm are represented by corresponding shades of gray and black, and very great care has been exercised in trying to represent comparable degrees of color intensity in the same way in all drawings. After Helly's fixation the red color seems localized in coarse fibrous strands, or sometimes in granules, but after aceto-carmine or Navashin's fluid the basophilic material is not associated with any particular cytoplasmic constituent.

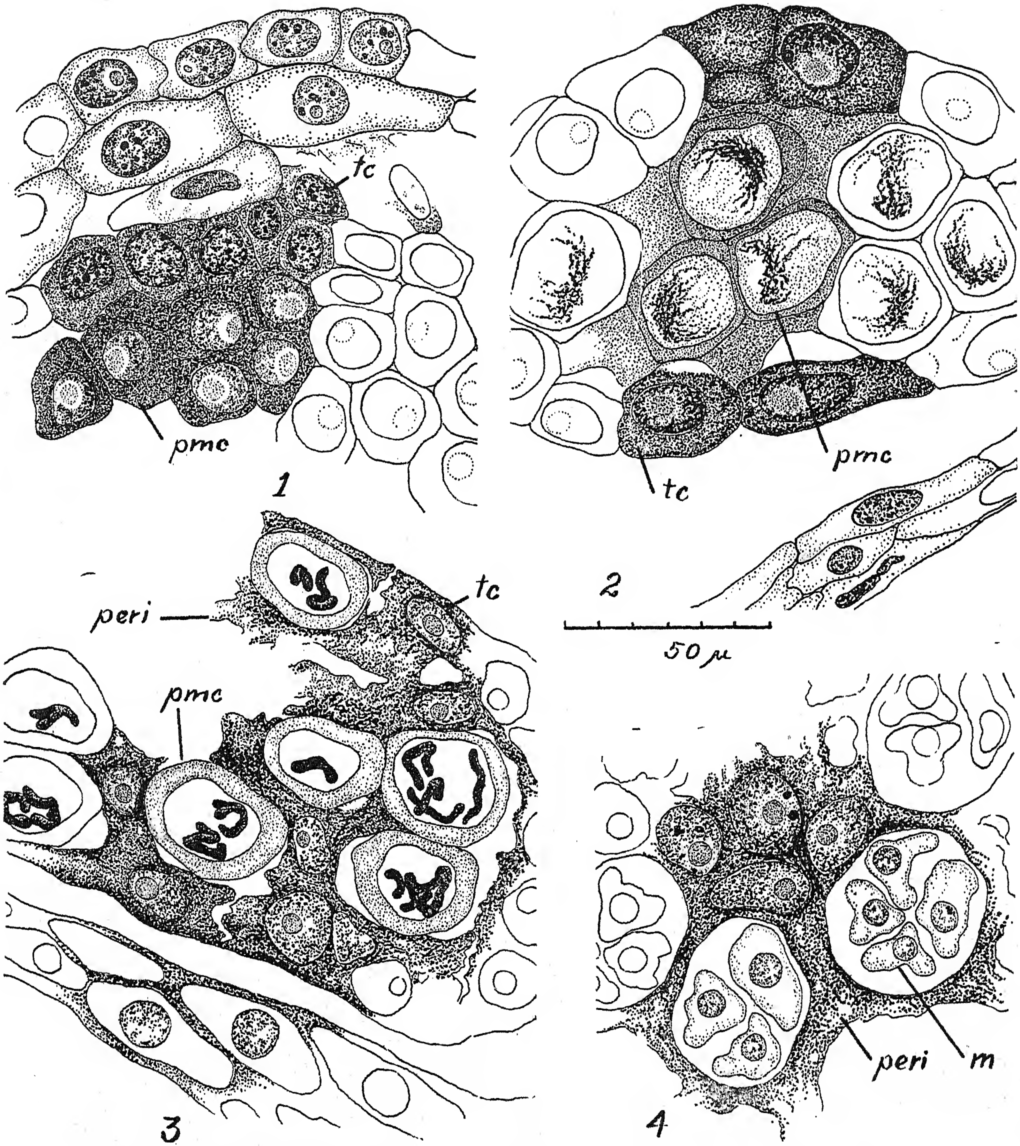
Observations

In general, the formation of pollen in *Rhoeo* is like that of other flowering plants. On the inside of the anther wall there is a single layer (usually) of tapetal cells which surrounds the pollen mother cells. In many plants the tapetal cells remain attached to the anther wall and undergo dissolution as the pollen develops, so that the latter is bathed in what has been commonly regarded as a nutritive fluid. In *Rhoeo*, however, the tapetal cells become detached from the anther wall first, and then, about the time of diakinesis in the pollen mother cells, the cell walls disappear in the tapetal tissue and a multinucleate syncytium or periplasmodium results. The latter is absorbed by the maturing pollen grains.

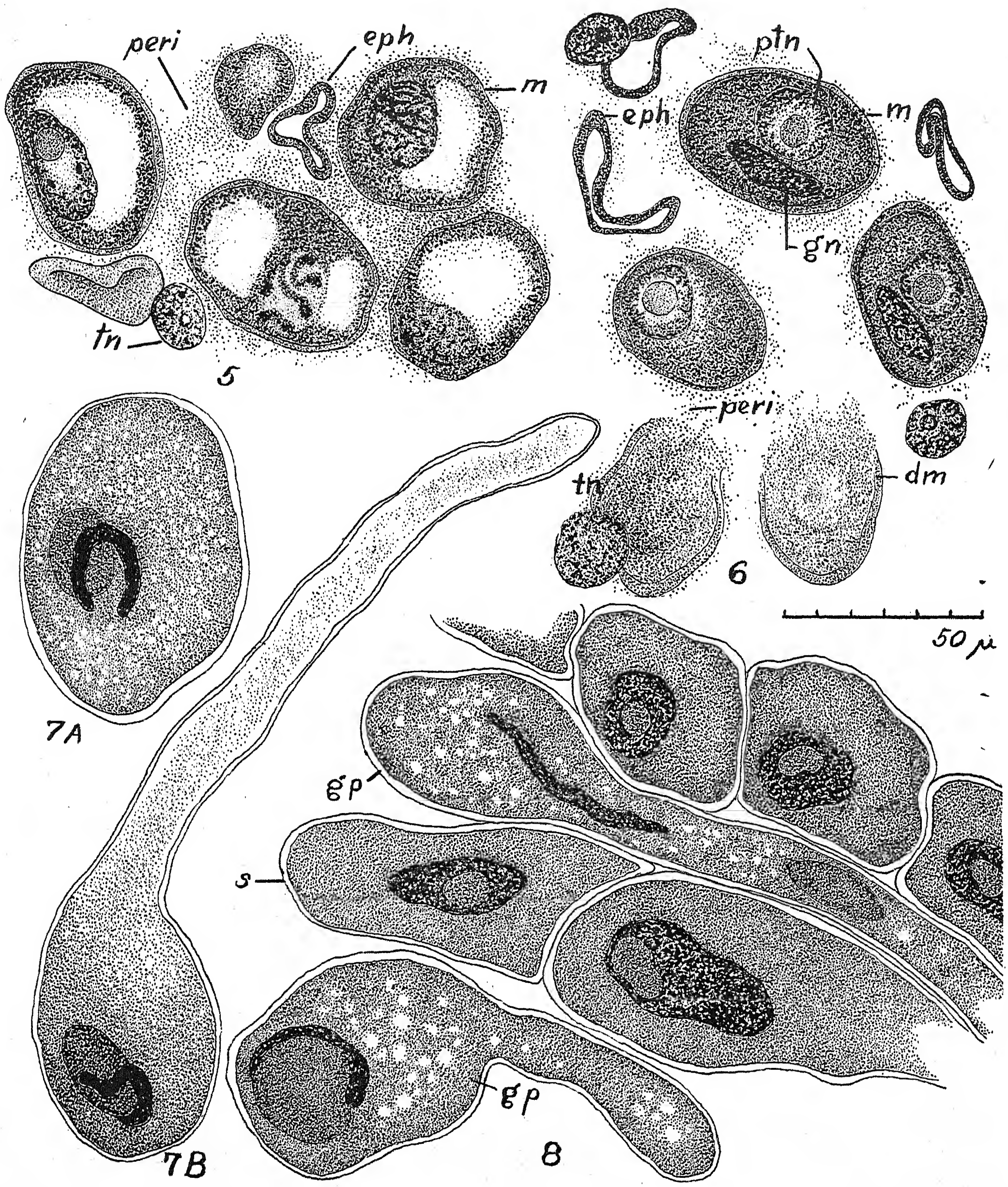
Meiotic growth.—Figures 1–3 are sections through anthers showing several phases of this growth period. Three salient points are brought out by the drawings. First is the increase in the volume of the pollen mother cells (*pmc*). Second, in the earlier stage (fig. 1), the cytoplasm of these cells stains an intense red, but as growth goes forward this basophily almost or entirely disappears (fig. 3). In contrast, the cytoplasm of the tapetal cells (*tc*) retains its basophily throughout this period. Third, at about the stage represented in figure 3, the walls of the tapetal cells disappear and some of the tapetal nuclei show a loss of chromaticity, that is, are beginning to undergo dissolution.

During the two meiotic divisions the cytoplasm of the pollen mother cells is almost or entirely free of basophilic materials, and the four microspores which result occupy about the same space as the single pollen mother cell from which they came (fig. 4). That is, there is no appreciable growth in total volume during this period. After fixation with Helly's fluid the microspores usually appear shriveled, but after Navashin's fluid this artifact does not show.

Post-meiotic growth.—Figures 4–7 show steps in the growth of microspores.



FIGS. 1–4.—Fig. 1, section through young anther showing tapetal cells (*tc*) and pollen mother cells (*pmc*). Figs. 2, 3, sections through anthers showing two stages in growth of pollen mother cells before first meiotic division. Fig. 4, section showing very young microspores (*m*) immediately after the two meiotic divisions.



FIGS. 5–8.—Fig. 5, microspores (*m*) at stage when nuclei are preparing for division; tapetal nuclei (*tn*) and empty pollen hulls (*eph*) appear in periplasmodium (*peri*). Fig. 6, later stage in microspore growth showing normal pollen grains with pollen tube (*ptn*) and generative (*gn*) nuclei. Some microspores are degenerating (*dm*), and periplasmodial fluid is nearly all absorbed. Fig. 7*A*, *B*, mature and germinating pollen cells, respectively. Fig. 8, optical section through stigma showing thick-walled and very basophilic cells of stigma (*s*) and germinating pollen (*gp*) (from aceto-carmine whole mount).

At the outset (fig. 4) the cytoplasm of the microspores does not stain with pyronin or at most is a very light pink. Figure 5 is a later stage in which the microspores are much larger. This increase in size is due to growth in the size of the nucleus, to the volume of the cytoplasm, and to the development of a cell vacuole. At the same time the cytoplasm has become very basophilic. In the anthers studied, many of the microspores for some reason fail to develop completely; such cells undergo rapid dissolution, and empty cellulose hulls (*eph*) are a conspicuous feature in all the older anthers. The cytoplasm and many nuclei of the periplasmodium are largely dissolved by this time and, after fixation with Helly's fluid, give a fine fibrous coagulum which stains red to pink. In figure 5 several microspore nuclei show condensation of the chromosomes in preparation for the postmeiotic division, which will give the mature pollen grain two nuclei.

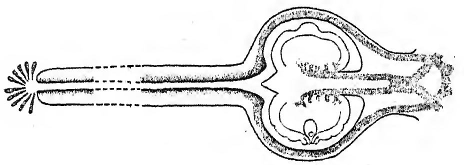


FIG. 9.—Semi-diagrammatic section through pistil showing distribution of cells which exhibit intense basophily of cytoplasm.

In figure 6 two normal pollen grains are shown. The cytoplasm has increased greatly in these cells, so that the cell vacuole is almost or wholly obliterated, and it stains deeply with pyronin. By this time most of the fluid of the periplasmodium has been absorbed and only a few tapetal nuclei remain. A fully mature pollen cell is shown in figure 7*A*. Such cells have increased in volume (as compared with the stage shown in figure 6), the generative nucleus has become greatly elongated, is often U-shaped, and the cytoplasm contains a number of round, light-refracting granules.

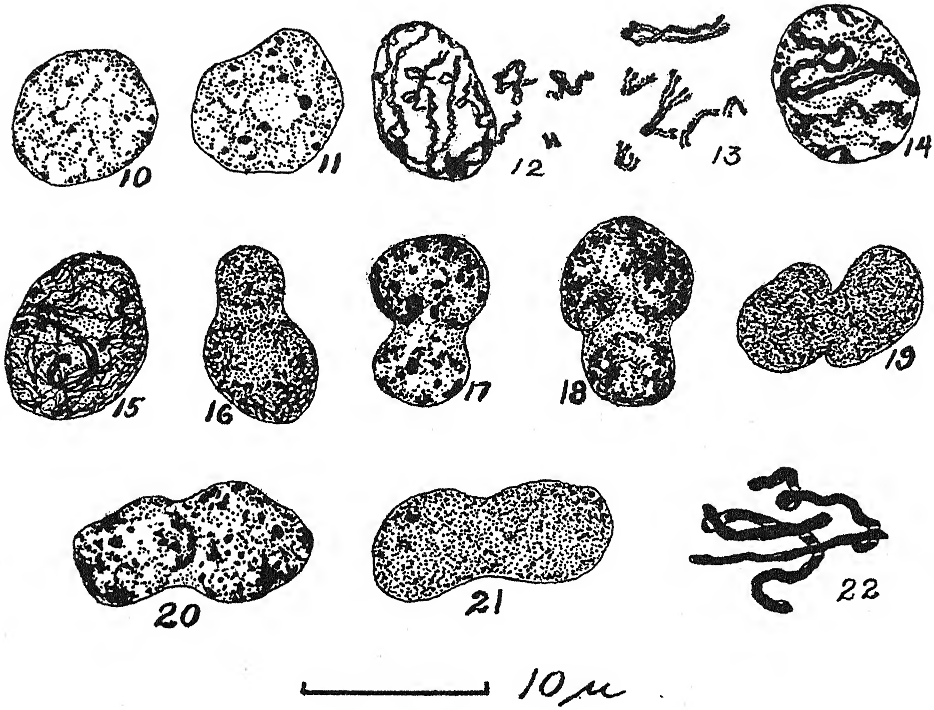
POLLEN-TUBE GROWTH.—When mature pollen grains are placed on the stigma of the flower they increase somewhat in volume and the cytoplasm stains less intensely. As the pollen tube is formed and penetrates the space between the cells of the stigma, its cytoplasm stains a bright pink, and this basophily persists for a long distance down the lumen of the style (figs. 7*B*, 8). The total volume increase in the germinating pollen cells is estimated as two to four times the volume (of the cytoplasm) of mature pollen grains.

There are definite areas in the pistil of the flower which may show heavy deposits of ribonucleic acid in the cytoplasm of cells. These are shown semidiagrammatically in figure 9. The very large thick-walled cells at the surface of the stigma are about as basophilic as the mature pollen grains (fig. 8). A lumen passes from the loosely organized stigma down through the style to the ovary, where it divides so that each of the three carpels (only two shown) receives a branch. The pollen tubes thus have a passageway which gives them access to the micropyles of the ovules. The cells immediately surrounding the lumen in the style are small and the cytoplasm is very basophilic. Near the top of the ovary the basophilic tissue splits into three bundles which pass around the outer wall of the ovary to the pedicel. These bundles of tissue are closely associated with spiral vessels (not shown). A separate mass of basophilic cells arises in the stem and passes to the carpels, where numerous branches spread out into the tissue which is destined to form the greater part of the seed. The more exact details of the distribution of ribonucleic acid in the ovary are now being studied. Incidentally, oxalic acid crystals, often

present in the walls of the ovary, stain brilliantly with pyronin.

TAPETAL TISSUE.—In very young flower buds (fig. 1) the anther wall is composed of large vacuolate cells, the cytoplasm of which stains a light pink color with pyronin. Normal mitoses are often found. Although in the diploid tissues of *Rhoeo* two nucleoli are seen, one larger than the other, at the stage shown in figure 1, these are inconspicuous in the anther wall. Heterochromatic granules are always prominent; two pairs are quite large and there are a number of smaller bodies. As the anther grows the cells in the wall increase in number by mitoses and the cellulose walls become rather thick (fig. 3).

In the tapetal layer at the stage shown in figure 1, in contrast to the cells of the anther wall, the cells are much smaller, no cell vacuole is visible, there are much heavier deposits of chromatin in nuclei, and the cytoplasm stains deeply with pyronin. Nucleoli are either small or entirely absent at this time. As the pollen mother cells increase in size (fig. 2) some of the tapetal cells and their nuclei increase greatly also and nucleoli become prominent. Since growth of the pollen mother cells involves the reduplication of the chromosomes, the question arises: Is the growth of the tapetal cells accompanied by a similar doubling of the chromosomes? COOPER (9) has reported that in *Rhoeo* the nucleus may divide mitotically one or more times, and if the resulting nuclei do not fuse multinucleate cells



FIGS. 10–22.—Various endomitotic division stages in tapetal nuclei. Figs. 10, 11, resting stages. Figs. 12, 13, split and coiling prophase chromosomes. Figs. 14, 15, maximum degree of condensation of chromosomes observed. Figs. 16–21, constriction of nuclear wall following condensation of chromosomes. Fig. 22, normal somatic metaphase showing size of dividing chromosomes.

result. In the writer's material no multinucleate tapetal cells have been observed, nor has there been any case in which the nuclear wall has been broken down. On the other hand, various normal prophase stages are common, showing that the chromosomes are condensing by coiling, and dumbbell-shaped nuclei are frequent (figs. 10–21). Such figures suggest that, in addition to the normal mitoses and fusion observed by COOPER, tapetal nuclei also increase in volume by an intranuclear, or endomitotic, division cycle.

Discussion

The preceding observations are of general interest because of the light they throw on the questions: What is the immediate source of the nucleic acids which appear in pollen mother cells or their derivatives; What roles do cytoplasmic nucleic acids play in growing cells; What is the relation between cytoplasmic and nuclear nucleic acids?

At the outset it is desirable to have in mind some basic facts about nucleic acids. These are made up of nucleotides, each of which contains phosphoric acid linked with a definite type of sugar, which in turn is linked with a nitrogen-bearing purine or pyrimidine base. The conventional formula for ribose nucleic acid is the tetranucleotide:

Phosphoric acid–ribose sugar–adenine (purine)
Phosphoric acid–ribose sugar–cytosine (pyrimidine)
Phosphoric acid–ribose sugar–guanine (purine)
Phosphoric acid–ribose sugar–uracil (pyrimidine)

In animals and plants two types of nucleic acids are commonly found, desoxyribose and ribose, which differ chemically in a slight change in the sugar component. In chromatin, or thymonucleic, or desoxyribose nucleic acid, as it is variously called, an H is substituted for an OH group in the ribose sugar giving desoxyribose sugar. In addition to the difference in the sugar, chromatin differs from ribonucleic acid in the following ways: (*a*) No desoxyribose nucleotide is known which enters into the makeup of a coenzyme, whereas all nucleotides known to act as coenzymes have the ribose sugar; in this sense chromatin may be spoken of as an enzymatically inactive form of nucleic acid. (*b*) Desoxyribose nucleotides are easily polymerized into very large molecules, and the evidence from ultra-centrifuge and X-ray determinations indicates that chromatin has about 2000 such nucleotides linked together; evidence for such high polymers of ribose nucleotides is not well established. (*c*) Chromatin is normally confined to the nucleus, while ribose nucleic acids are predominantly cytoplasmic in location.

There is considerable direct and indirect evidence that in living cells of both plants and animals one form of nucleic acid is easily changed into the other; indeed, WHITE (19) has suggested that there is such an exchange during each mitotic cycle, a conclusion which seems to be supported by the present study on *Rhoeo*. MASING (10), using quantitative chemical methods, showed that there is as much nucleoprotein (measured as phosphorus combined with protein) in the unsegmented egg of the sea urchin as in the larva when it hatches, and from this he concluded that there had been no synthesis of nucleoproteins during cleavage. Later, BRACHET (1) demonstrated that the nucleoprotein of the unsegmented egg of the sea urchin is in the form of ribonucleic acid. During cleavage the amount of ribonucleic acid falls off as the quantity of thymonucleic acid increases, and BRACHET concluded that the ribose was partially converted into the desoxyribose type during cleavage.

That thymonucleic acid is readily changed into the ribose type is indicated by two lines of evidence. CASPERSSON

and SCHULTZ (7) found that there is less ribose nucleic acid in the eggs of XX females of *Drosophila* than in females of the XXY type, the extra nucleic acid being brought into the egg by the extra Y chromosomes of the nurse cells. Again, in the germinal vesicle of the eggs of frogs and toads, during the early growth of the oocytes granules of heterochromatin become detached from the chromosomes, migrate to the surface of the nucleus, and there act as centers for the elaboration of ribonucleic acid which accumulates in large quantities just outside of the nuclear wall. During this process the heterochromatin disappears, presumably being used up or somehow converted into the ribose form (3, 15, 16).

The meiotic growth period throws no light on the source of the ribonucleic acid in the cytoplasm of pollen mother and tapetal cells, because in the youngest anthers examined large amounts of this basophilic material are already present. The growth of the pollen mother cells at this time seems to center mostly in the nucleus, since the nucleo-cytoplasmic ratio shifts from about 1:2 to 1:1. In preparation for the meiotic divisions, the chromosomes are reduplicated once, perhaps twice, and the fact that during this period the ribonucleic acid disappears from the cytoplasm suggests that it is transferred to the nucleus and enters into the formation of the chromosomes as thymonucleic acid. On the other hand, in some of the tapetal cells the chromosomes must have been reduplicated, since some cells attempt to divide, but so far as can be determined the cytoplasm of such cells shows no decrease in basophily. It seems possible that in these cells—which are destined to undergo cytolysis—the nucleic-acid metabolism is disturbed, just as when proliferating cells are irradiated (11).

During the meiotic divisions, which go forward without an intervening resting stage, there is little or no basophilic material left in the cytoplasm, and the very young microspores contain practically none of this material. Concomitant with the onset of growth in the microspores, ribonucleic acid appears in the cytoplasm, and—if the degree of basophily is a reliable index—its concentration steadily increases and reaches its maximum in the mature pollen grains. Where does this nucleic acid come from? Either it is derived from the chromatin of the microspore nucleus or it is synthesized from the fluid in which the microspores are bathed. The first seems unlikely, since after the two meiotic divisions the microspore nucleus is less chromatic than at any other period. On the other hand, the fluid in which the microspores are bathed is derived from the dissolution of tapetal cells and of microspores which fail to complete development, and both of these sources are very rich in ribonucleic acid. Although little is known as yet about the steps involved in the transfer, that is, how far the polymerized ribose nucleotides are broken down, it seems a safe inference that the anther fluid is the source of the nucleic acids which accumulate in the cytoplasm of microspores which become mature.

If ribonucleic acid is essential for growth, why is so much of it present in mature pollen grains when growth is at a standstill? In answering this question it may be pointed out that the history of the tapetal cells and of the development of pollen has a close parallel in animal eggs formed through the agency of nurse cells, and fundamentally the ends subserved seem the same in both cases. In *Drosophila*, for example, the oocyte grows at the expense of fifteen nurse cells. The latter become very large, are high polyploids containing large amounts of chromatin and much ribonucleic acid

(14, and unpublished data). By the time the oocyte becomes mature the substances in the nurse cells have been completely absorbed in the egg cytoplasm, which is now rich in ribonucleic acid. In the same way in *Rhoeo*, as the pollen matures, the tapetal and degenerating germ cells are completely absorbed. Apparently the reason so much nucleic acid is deposited in the cytoplasm of animal eggs is that it is needed for the rapid synthesis of proteins and other complex substances as the oocyte and embryo develop and as a source of nucleic acids for the formation of cleavage nuclei (13). Similarly, in the case of the pollen in *Rhoeo*, large amounts of cytoplasmic nucleic acid are needed for the extremely rapid elaboration of protoplasm during the formation of the pollen tube.

It seems possible that the abundant ribonucleic acid in the style of the flower along the path which the pollen tube follows insures an ample supply of food material for the growing pollen tube. The basophilic tissue which passes from the pedicel of the flower into the carpels, where rapid synthesis of new materials takes place, emphasizes the relation between growth and the presence of ribonucleic acid, although it is not clear why cells far removed from regions of most rapid growth should be so basophilic. It is significant, perhaps, that these cells are associated with the spiral vessels which carry water to the growing tissues and suggests that other substances may be transported.

While it is clear that ribonucleic acid is essential for cell growth, the way it functions is not well understood. It has long been recognized that enzymes play dominant roles in the chemistry of the cell. And the fact that so many of the known enzymes contain ribose nucleotides has led a number of workers to suggest that the origin of these are the cytoplasmic nucleic acids so abundant in rapidly synthesizing cells. This explanation receives support from the fact that a nucleotide containing adenine is found in coenzymes 1 and 2 and adenylic acid. OSTERN, TERSZAKOWEC, and HUBL (12) were able to change ribonucleic acid into adenosine, *in vitro*, and have interpreted their results as a step in the formation of adenylic acid. CASPERSSON and BRANDT (6) point out that, while probably (though as yet unproved) coenzymes come from ribonucleic acids, there is far more of the latter present in the cytoplasm than would be needed to catalyze chemical reactions, and they think the ribose nucleotides play other fundamental roles in growth. There is one possibility which has not yet been stressed.

Since nucleic acids were first found in nuclei it is natural to consider new nucleotides as being synthesized at this site in the cell. On the other hand, many facts about the mitotic cycle can best be understood by assuming that new nucleotides are synthesized first in the cytoplasm and are then passed somehow into the nucleus. On this assumption a mitotic cycle might involve the following changes. During the resting stage CASPERSSON (4) found that the total amount of nucleic acid in the nucleus is at a minimum, whereas it is known (17) that cytoplasmic activity including growth and metabolism is at a peak, presumably because of the presence of ribose nucleotides which are essential for growth. When growth is complete and the prophase begins, ribose nucleotides pass into the nucleus and are there converted into desoxyribose nucleotides and carried in a compact, highly polymerized form on the chromosomes over into the two daughter cells. This migration of nucleotides (however it may occur) into the nucleus ex-

plains the sudden increase in nucleic acid which CASPERSSON observed in the nucleus, a fact which in the past has been interpreted to mean that new nucleotides were being synthesized in the nucleus from relatively simple components. During the pro-metaphase stages cytoplasmic activity is at a low ebb (17), presumably because of the absence of ribonucleotides (coenzymes). After the telophase, the decrease in the amount of nucleic acid in the nucleus is due to the passage of some of it into the cytoplasm, where it is converted into ribose nucleotides, there to implement protein growth and the synthesis of new nucleotides, and perhaps to perform other functions not now recognized.

This view of nucleotide exchange between nucleus and cytoplasm is (except for minor details) essentially that expressed by WHITE (19) and is supported by evidence from diverse fields of investigation. SCOTT (18), studying mitosis by the microincineration method, found that in the resting stage the inorganic ash, consisting mainly of calcium and magnesium residues, was evenly distributed in the cell. In the prophase of mitosis these salts pass into the nucleus and are deposited on the chromosomes. The simultaneous passage of nucleotides and of calcium and magnesium ions into the nucleus suggests that the latter may play some role in the attachment of these nucleotides to the protein framework of the chromosomes.

MITCHELL (11) has found that when growing and differentiating cells are irradiated with X- or gamma rays, during the period when mitoses are inhibited, there is an accumulation of ribose nucleotides in the cytoplasm of the cells. Quantitative measurements indicate local concentrations of as much as 3 per cent. During this time there is no increase in the amount of nucleic acid in the nucleus. MITCHELL explains these facts as "probably due to inhibition by the radiation of the process of reduction of ribonucleotides to desoxyribo-nucleotides in the nucleus."

If ribose nucleotides of the resting stage are in part precursors of chromatin in rapidly growing and dividing cells, this would account for some of their concentration in the cytoplasm. Furthermore, as in other organic systems, the two types of nucleic acid would be expected to be in some sort of equilibrium, and this would explain the presence of large amounts of chromatin in the nucleus when the cytoplasm shows large amounts of ribose nucleotides.

Attention should be drawn to the limitations of the methods employed in this study. Basophily in the cytoplasm depends on the presence of free acid groups, and oxalic-acid crystals, which often show in the walls of the ovary in *Rhoeo*, stain as brilliantly as the ribose nucleotides. When the basophily is removed from the cytoplasm by treatment with the ribonuclease enzyme, the conclusion is warranted that polymerized ribose nucleotides were present initially. The absence of basophily in the first place, however, does not indicate necessarily the absence of ribonucleotides but the absence of ribonucleotides with uncombined or free acid groups. Obviously the methods employed are useful only in species in which the ribonucleic acid can be stained with basic dyes.

Summary

1. A cytoplasmic basophily, which is removed when cells are treated with a ribonuclease enzyme, has been used as a means of identifying ribose nucleic acids and of following their distribution in the cells of the anthers of *Rhoeo*, and to a

lesser extent in the pistil of the flower. Rapid cell growth is invariably accompanied by large amounts of ribonucleic acid in the cytoplasm and heavy deposits of chromatin in nuclei.

2. In young anthers the cytoplasm of both tapetal and sporogenous tissues is extremely rich in ribonucleic acid. As the pollen mother cells prepare for the meiotic divisions, the ribonucleic acid disappears from the cytoplasm, presumably being passed into the nucleus and changed into desoxyribose nucleic acid (chromatin). Initially the cytoplasm of microspores is devoid of basophilic material, but as they grow into mature pollen grains large amounts of ribonucleic acids accumulate in the cytoplasm.

3. The growth of tapetal cells is accompanied by one or more endomitotic nuclear division cycles. The tapetal cells, which are rich in cytoplasmic nucleic acid at the time of cytolysis, are the source of the ribonucleic acid which accumulates in the maturing pollen grains.

4. There is a similarity between microspore growth at the expense of tapetal cells and growth of oocytes in animals through the agency of nurse cells. Large amounts of ribonucleic acid are stored in mature pollen grains and used in the extremely rapid synthesis of new materials which accompanies pollen tube growth.

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LITERATURE CITED

1. Brachet, J., Recherches sur la synthèse de l'acide thymonucléique pendant le développement de l'œuf d'Oursin. Arch. Biol. 44:519–576. 1933.
2. ———, La detection histochimique des acides pentosenucléique Comp. Rend. Soc. Biol. 133: 88–90. 1940.
3. ———, La localisation de l'acide thymonucléique pendant l'oogenèse et la maturation chez les Amphibiens. Arch. Biol. 51:151–165. 1940.
4. Caspersson, T., Ueber den chemischen Aufbau der Strukturen des Zellkernes. Skand. Arch. Physiol. Suppl. No. 8. 73:1–151. 1936.
5. ———, Methoden zur physikalischen Analyse der Zellstruktur. Fortschr. Zool. N.F. 2:270–284. 1937.
6. Caspersson, T., and Brandt, K., Nucleotidumsatz und Wachtum bei Presshefe. Protoplasma 35:507–526. 1941.
7. Caspersson, T., and Schultz, J., Nucleic acid metabolism of the chromosomes in relation to gene reproduction. Nature 142:294–295. 1938.
8. ———, and ———, Ribonucleic acids in both nucleus and cytoplasm and the function of the nucleolus. Proc. Nat. Acad. Sci. 26:507–515. 1940.
9. Cooper, D. C., Nuclear divisions in the tapetal cells of certain angiosperms. Amer. Jour. Bot. 20:358–364. 1933.
10. Masing, E., Ueber das Verhalten der Nucleinsäure bei der Furchung des Seeigleies. Zeitsch. physiol. Chem. 67:161. 1910.
11. Mitchell, J. S., Disturbance of nucleic acid metabolism produced by therapeutic doses of X and gamma radiations. III. Inhibition of synthesis of thymonucleic acid by irradiation. Brit. Jour. Exp. Path. 23:308–314. 1942.
12. Ostern, P., Terszakowec, J., and Hubl, St., Über die Phosphorylierung des Andenosins durch Hefe und die Bedeutung dieses Vorgangs für die alkoholische Gärung. III. Bildung des Adenosins und Zerfall der Nucleinsäure in der Hefe. Zeitsch. physiol. Chem. 255:104–125. 1938.
13. Painter, T. S., On the synthesis of cleavage chromosomes. Proc. Nat. Acad. Sci. 26:95–100. 1940.
14. Painter, T. S., and Reindorp, E., Endomitosis in the nurse cells of the ovary of *Drosophila melanogaster*. Chromosoma 1:276–283. 1939.
15. Painter, T. S., and Taylor, A. N., Nuclear changes associated with the growth of oocytes in the toad. Anat. Rec. Suppl. 78:84. 1940.
16. ———, and ———, Nucleic acid storage in the toad's egg. Proc. Nat. Acad. Sci. 28:311–317. 1942.
17. Peter, K., Zellteilung und Resorption. Beobachtungen an normalen Nieren. Zeitsch. Anat. Entw. 72:463–486. 1924.
18. Scott, G. H., The deposition of the fixed mineral salts during mitosis. Bull. histol. Appliq. 7:251. 1930.
19. White, M. J. D., Nucleus, chromosomes, and genes. Chap. V. Cytology and cell physiology. Oxford Press. 1942.

HISTOLOGICAL COMPARISON OF CUCUMBER FRUITS DEVELOPING PARTHENOCARPICALLY AND FOLLOWING POLLINATION

CONTRIBUTIONS FROM THE HULL BOTANICAL LABORATORY 554

J. O. YOUNG

Introduction

Parthenocarpy in the cucumber has long been recognized by horticulturists, but it was first made the subject of botanical investigation in 1902 by NOLL (15), who defined the term and observed fruits containing abortive seeds with undersized seed coats. Two years later KIRSCHNER (13) reported a doubtful case in which 78 out of 95 seeds produced in a single unpollinated cucumber were viable. HARTLEY (12) reported the stimulation of fruit development in tobacco by treating the stigmas with *Azalea* pollen, corn flour, magnesium sulphate, or air-slaked lime. FITTING (4), YASUDA (20), and GUSTAFSON (10) have achieved similar results with pollen extracts.

Since the development of the growth-substance concept, several investigators, notably GUSTAFSON (8, 9), GARDNER and MARTH (6), and WONG (19), have succeeded in inducing fruit development by growth-promoting chemicals. These investigators worked chiefly with species of the Cucurbitaceae and Solanaceae, as well as with holly and strawberry. GARDNER and KRAUS (5) made a histological study of artificial parthenocarpy in the holly, noting that development closely parallels that of pollinated fruits, except for the disintegration of the megagametophyte and the absence of embryo and endosperm. VAN OVERBEEK *et al.* (18) reported the formation of pseudo-embryos in *Datura* fruits which had been injected with auxin.

TUKEY (17) showed that living embryos are essential for development of cherry and peach fruits. He correlated his findings with the three stages in the growth curves of these drupe fruits. Using the *Avena* test method, GUSTAFSON (7, 11) reported that auxin concentration of fruits is highest in developing ovules and associated tissues, and that it is higher in normally seeded than in parthenocarpic fruits of the same species. He concluded that auxins play a major role in fruit development.

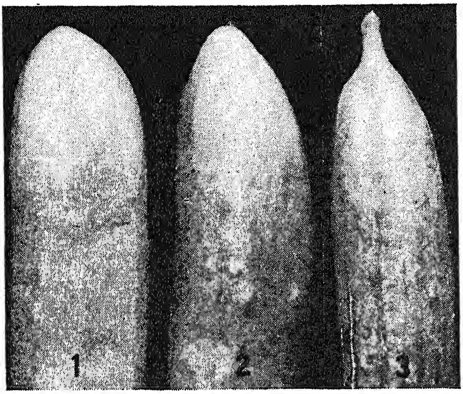
Certain varieties of cucumber regularly produce fruits without pollination. The results obtained with the variety Rollison's Telegraph, which exhibits natural parthenocarpy, are given here.

METHODS.—Seeds were planted February, 1941, in rich garden soil and grown on a well-lighted greenhouse bench. The plants were kept watered at all times but no attempt was made precisely to control the temperature or the humidity. During the later stages of the experiment the plants were supplied with complete nutrient solution high in nitrate to insure continued vigorous growth. The vines were trained upon a horizontal trellis about 7 feet above the floor level, and the developing fruits were allowed to hang without support.

Uniform pistillate flowers were selected at two developmental stages and treated with 2 per cent indoleacetic acid in lanolin paste. Controls were treated with pure lanolin. One lot was treated at full bloom and a second lot at approximately 4 days before full bloom. At the

time of anthesis the ovaries used ranged from 3.5 to 4.5 cm. in length. Larger or smaller ovaries were discarded. Four days before full bloom the developing ovaries were 1.8–2 cm. long and the still-green petal lobes extended about 4 mm. beyond the calyx.

The upper portion of the perianth, including the stigma and staminodia, was removed by a transverse cut through the nectary, and the indoleacetic acid in lanolin paste was spread in a thin layer over the freshly exposed surface. The lower part of the floral tube remained attached to the ovary by the narrow neck about 1 mm. long. Pure lanolin was used for controls. A third lot of flowers was hand-pollinated, and a fourth was allowed to develop without treatment.



FIGS. 1–3.—Apical portions of fully developed fruits: Fig. 1, pollinated; untreated fruits and controls treated at full bloom were similar in appearance. Fig. 2, treated with indoleacetic acid at full bloom; fruits treated with pure lanolin 4 days before full bloom were similar in appearance. Fig. 3, treated with indoleacetic acid 4 days before full bloom, showing apical tumor.

Portions of the fruits were fixed in Navashin's fluid and transferred to paraffin by the tertiary butyl-alcohol method. The material was transferred from the fixing fluid directly to a mixture containing ethyl alcohol 50 per cent, tertiary butyl-alcohol 20 per cent, and water 30 per cent. This procedure gave as good results as the more laborious method of washing in water and running through a longer series of changes. Sections were cut at 10 μ and stained in safranin, gentian violet, and orange G.

Gross development

Virtually all the ovaries, including those untreated, developed rapidly for several days. Toward the end of the experiment, however, the presence of large developing fruits inhibited early growth of other fruits on the same vine. Fruits treated with the acid 4 days before full bloom were decidedly smaller than any of the others.

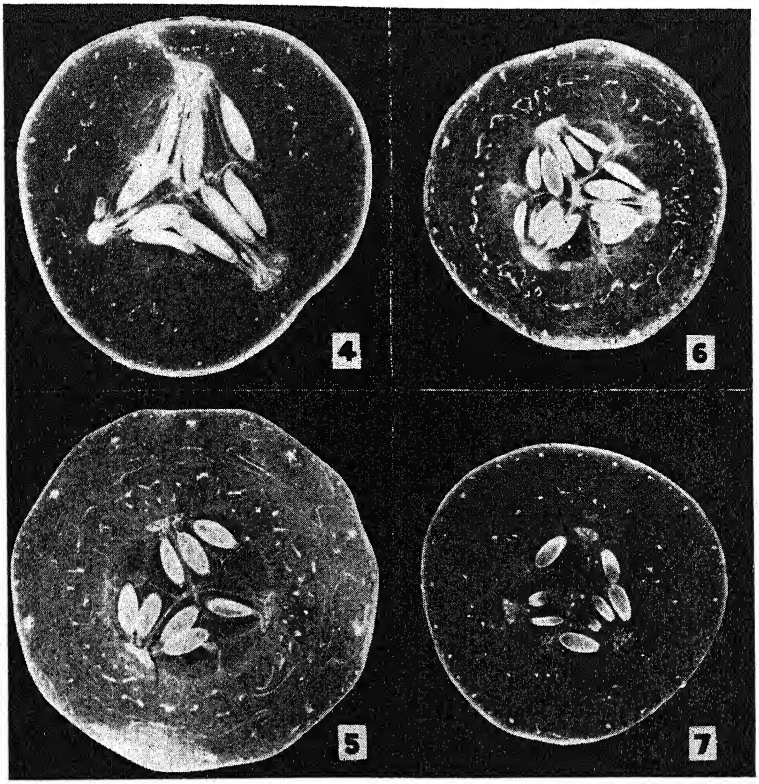
POLLINATED FRUITS.—Within a day or two after pollination the perianth and included structures began to wither, and usually they were completely dried within a week. These dead tissues usually fell away and left a smooth rounded scar (fig. 1). At the end of 20 days many of the ovules had attained a length of 12 mm. and the hardened seed coats inclosed well-formed embryos (fig. 4).

UNTREATED FRUITS.—In external appearance the untreated fruits developed identically with the pollinated fruits. Some of the ovules attained full size, but more frequently they were slightly smaller (fig. 5) and the seed coats were often sunken on the flattened sides. In no cases were embryos found.

FRUITS TREATED AT FULL BLOOM.—Fruits treated with indoleacetic acid at full bloom differed from the untreated controls in that the apical tissues below the cut surface remained alive until harvested and sometimes increased slightly in size, resulting in a small protuberance at the tip of the mature fruit (fig. 2). The ovules were slightly smaller than those of the untreated fruits (fig. 6). In corre-

sponding controls treated with pure lanolin the neck and perianth tissues dried out in the same manner as those on pollinated or untreated plants.

FRUITS TREATED 4 DAYS BEFORE FULL BLOOM.—Fruits treated with indoleacetic acid 4 days before full bloom developed characteristic apical tumors, involving the tissues of the nectary, floral tube, and the neck. The tumors reached a maximum diameter of slightly more than 1 cm. and a length of nearly 2 cm. (fig. 3). At the time of treatment the nectary tissue was light yellow and the lower part of the floral tube only slightly green. During the first 2 days after treatment the tissues of the floral tube and neck became lighter in color and a slight swelling was observed. After 4–5 days the tumors began to develop a bright green color throughout. Subsequent growth of the tumors was more rapid, and maximum size was usually attained in 10–15 days. The tissues of the nectary proliferated more extensively than those of the floral tube and greatly overtopped the latter structure. In no case did the tumors develop the large irregular masses reported for stems and fruits of the bean (14). No root primordia were observed. Ovules attained a final size decidedly smaller than those of the controls (fig. 7). Hardening



FIGS. 4–7.—Cleared cross sections of fully developed fruits: fig. 4, pollinated, showing embryos; fig. 5, untreated; fig. 6, treated with indoleacetic acid at full bloom; fig. 7, treated with indoleacetic acid 4 days before full bloom.

of the integuments was markedly inhibited and restricted to the micropylar end of the ovule.

The apical portions of controls treated with pure lanolin at 4 days prior to full bloom remained intact for several days. At the end of 24 days, tissues of the floral tube, nectary, and part of the neck were dead but no periderm or scar tissue had formed. At this time the gross appearance was similar to that of fruits treated with indoleacetic acid at full bloom (fig. 2), except that there was more dead apical tissue.

Histological details

FERTILIZED OVULES.—No comprehensive published account of ovule development in the cucumber has been found. TILLMAN (16) investigated the megagametophyte development and embryology. She noted the massive nucellus, the swelling of the pollen tube, digestion of cells within the nucellar beak, and the attainment by the ovules of a strictly anatropous position. The findings of the writer are similar in all major respects. FICKEL (3) showed that the major portion of the seed coat is derived from the outer integument, the inner integument attaining a thickness of only two cell layers. BARBER (1) added a few details of cell size and arrangement but erroneously figured an inner integument several cells in thickness early in development.

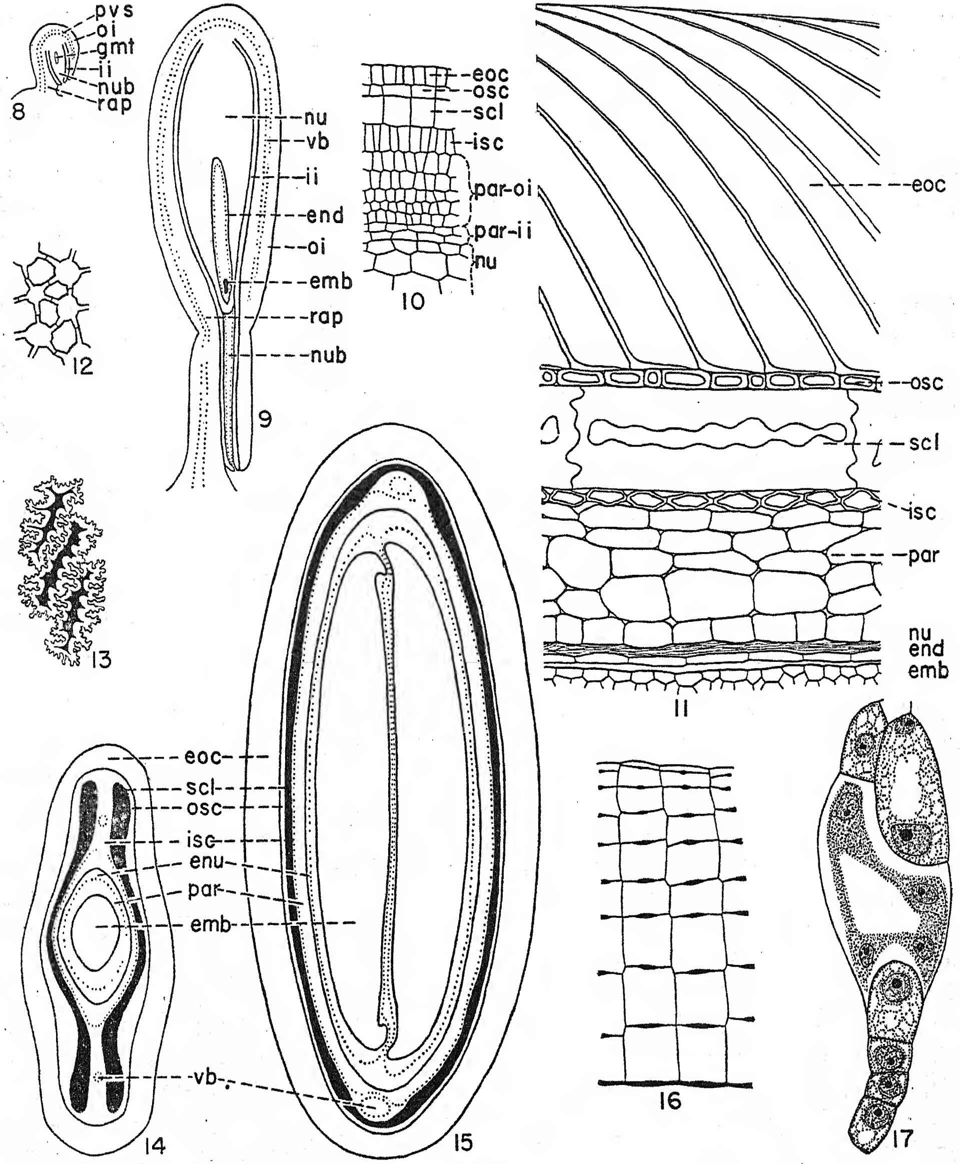
Four days before full bloom the megaspore mother cell may be clearly distinguished two to four cell layers from the tip of the nucellus. Continued tangential division of the overlying cells results in the development of a long nucellar beak, which is discernible at the time of anthesis. The inner integument develops as a covering two or three cells in thickness, which finally closes over the nucellar beak. The outer integument arises as a more massive layer and may be eight cells in thickness by the time of full bloom. A vascular strand extends through the raphe to the chalaza and is continuous with a strand which extends through the outer integument as far as the base of the nucellar beak (fig. 8).

At full bloom meiosis has been completed and the gametophyte is partially or fully developed. It occupies a position just proximal to the base of the nucellar beak. At this stage mitotic activity is evident in the integuments and at the chalazal end of the nucellus.

Fertilization is accomplished in ovules near the apical end of the ovary within 72 hours after pollination. Just before fertilization the pollen tube enlarges in the proximal portion of the nucellar beak and nucellar cells in this region are digested. Soon after fertilization, cells of the nucellus adjacent to the embryo sac become meristematic, and several mitotic divisions occur. Eight days after full bloom the embryo is a small, slightly elongated cellular mass (fig. 9). At this stage mitotic activity is still in progress but all tissues of the seed coat are clearly defined.

Subsequent development of the seed coat involves differentiation and maturation of the several tissues. Comparison of figure 10 (8 days from full bloom) and figure 11 (20 days after full bloom) brings out the development of tissue types in the seed coat.

The seed coat may be compared to a rigid laminated sac of many layers. In the mature seed, cells of the innermost layer are parenchymatous. This tissue is of mixed origin. The inner portion derives from the inner integument and the remainder arises from the inner four or five tiers of cells of the outer integument. As the seed coat matures the exterior tissues increase in size more than the interi-



FIGS. 8–17.—Fig. 8, longisection of ovule at full bloom; $\times 35$. Fig. 9, same, 8 days after full bloom; $\times 35$. Fig. 10, portion of longisection through seed coat 8 days after full bloom; $\times 350$. Fig. 11, same, 24 days after full bloom; $\times 350$. Fig. 12, stellate cells from tissue surrounding vascular bundle; $\times 450$. Fig. 13, elongated stone cells from macerated seed coat; $\times 200$. Fig. 14, cross section of mature seed near hilum; $\times 35$. Fig. 15, same, through center of seed; $\times 35$. Fig. 16, cells of outermost layer of seed coat as seen in cross section through seed, showing thickenings on cell walls; $\times 350$. Fig. 17, anomalous development of megagametophyte: four cells at lower part of figure are at micropylar end; $\times 750$ (*emb*, embryo; *end*, endosperm; *enu*, endosperm and nucellus; *eoc*, elongated outer cells; *gmt*, megagametophyte; *ii*, inner integument; *isc*, inner layer of thick-walled derivatives of stellate cells; *nu*, nucellus; *nub*, nucellar beak; *oi*, outer integument; *osc*, outer layer of thick-walled derivatives of stellate cells; *par*, parenchyma; *pvs*, provascular strand; *rap*, raphe; *scl*, elongated stone cells; *vb*, vascular bundle).

or parenchyma. The interior cells thus become flattened and are pulled from their original positions, so that at maturity the parenchymatous layer is about five cells in thickness. Intercellular spaces are formed and the identity of the inner integument as a distinct tissue is lost.

External to the parenchyma is a layer of stellate cells. This layer is composed of a single tier of cells on the flattened sides of the seed, but on the lateral margins it is many cells in thickness. The vascular bundle which extends around the seed is imbedded within this marginal layer of stellate cells. In the region of the vascular bundle little growth occurs and the stellate condition becomes best developed (fig. 12). At maturity each cell has many projections which extend in various directions and abut on adjacent projections from other cells. The tissue thus formed is lattice-like in appearance, the large intercellular spaces being merged to form a continuous three-dimensional labyrinth. Where this tissue is only one tier in thickness the cells become flattened as the seed coats enlarge. Thickening and hardening of the cell walls begins after the twelfth day from full bloom. This is first evident close to the base of the nucellar beak and proceeds rapidly to the chalazal end of the seed. No sclerenchyma is developed within the nucellar beak.

Outside the stellate cells is a layer of large stone cells which are greatly elongated in the longitudinal plane of the seed axis. Examination of macerated tissue shows these cells to be roughly rectangular in surface view, but the margins are deeply and irregularly incised (fig. 13). The lumen of each cell is narrow and branched and thus appears to be discontinuous when the seed is cut in median longitudinal section. In a few cells, however, the continuity of the lumen is clearly discernible (fig. 11). The cells of this tier are tightly dovetailed together, forming a hard impermeable layer. Near the nucellar beak this layer does not extend completely around the seed (fig. 14). In this region the tissue is in the form of two bands over the flat sides of the seed but not around the margins. Lateral to the vascular bundles this layer is two to four cells in thickness. The discontinuity of this layer around the hypocotyl permits the emergence of the embryo at germination when the empty seed coats remain intact.

The next layer is another tier of stellate cells. In the distal end of the seed coat this tissue is continuous with the inner layer of stellate cells (fig. 14). A cross section through the center of the seed, however, shows that here there are two layers of stellate cells separated by the tier of large stone cells already described (fig. 15). In the outer layer the stellate condition is practically lost when the cell walls thicken, but the various stages show that these cells are derived from the stellate type.

The outermost layer of the seed coat is composed of very elongate cells with characteristic thickenings on the inner radial walls. These cells are radially elongated 8 days after full bloom. As lengthening and maturation continue they become appressed, with the outer ends lying in the direction of the chalaza. Cross sections through the seed show that these cells develop in an orderly spatial arrangement. Since these cells overlap for most of their length, it appears in cross-sectional view that there are several distinct layers (fig. 16).

The elongated cells of this outer layer are continuous over the beak but lack thickenings in this region. The remainder of the beak is composed of a few stellate

cells and parenchyma. A constriction occurs at the base of the beak, and when the seed is broken away a considerable portion of the raphe, integuments, and nucellar remnants is lost (fig. 9).

In the mature seed the nucellus has been reduced to a membrane of collapsed cell walls, and a single layer is all that remains of the endosperm.

Unfertilized ovules.—Many stages in meiosis and megagametophyte development were carefully observed, but no deviations from the normal were found subsequent to treatment with indoleacetic acid, even when the application was made before the onset of the meiotic process. Three days after full bloom, however, the absence of the pollen tubes in ovules of unpollinated fruits was noted and the cells of the nucellar beak were not digested. No renewal of meristematic activity in nucellar cells adjacent to the megagametophyte occurred, as is the case when young embryos are present. In other portions of the ovule, divisions continued until 8 days after full bloom, or about as long as they do following fertilization.

The megagametophytes of the parthenocarpic fruits were occasionally observed to enlarge considerably. This sometimes continued for as long as 8 days after full bloom, but more often degeneration began about the fifth day. The fusion nucleus and the egg were usually the last recognizable remnants of the megagametophyte. These were sometimes discernible 16 days after full bloom. Even in the absence of an embryo, the nucellus developed into a massive tissue which completely filled the integuments. The cavity left by the degenerating gametophyte was often extended by cellular disintegration in the center of the nucellus.

Proliferation of gametophytic cells was observed in a single case. In a section of an ovule fixed 8 days after prebloom treatment with indoleacetic acid the condition shown in figure 17 was clearly seen. The free nuclear tissue is suggestive of endosperm and apparently has arisen by divisions of the fusion nucleus. The four cells at the micropylar end of the figure may have been derived from the egg. Since the antipodals have been observed to degenerate early in this variety, it would seem most probable that the two large cells at the chalazal end are of nucellar origin.

In the ovules of all fruits treated with indoleacetic acid and in the controls cell division continued long enough so that the pattern of the mature seed coats was definitely established. When the acid was applied 4 days before full bloom, hardening of the seed coats was greatly reduced and usually did not extend more than halfway to the chalazal end of the seed. When treatments were made at full bloom, hardening proceeded somewhat further, while in the controls the integuments sometimes reached a stage of development nearly equal to that of ovules containing embryos.

The preceding description is based on the most completely developed ovules, which usually are found near the apical end of the fruit. As a rule the ovules near the stem end are progressively smaller, and many of them never exceed 1–2 mm. in length.

Apical tissues.—Following pollination, the stylar canal tissue is digested or disrupted within 48 hours by the developing pollen tubes. The adjacent intact cells become elongated in a radial direction, but meristematic activity is not initiated in any part of the floral tube, nectary, stylar tissue, or neck. There was some increase in the thickness of cell walls in the peripheral parenchym-

atous cells of the neck at 4 days after full bloom. By this time the nectary tissue has degenerated and a periderm may have begun to form at the base of the neck. At 8 days after full bloom the periderm is well formed, and on its outer surface cell walls are appreciably thickened. All the distal dried-out tissues may now fall away, or they may remain attached. At 20 days after full bloom pitted stone cells were found abundantly in the tissue immediately beneath the apical periderm. Apical tissues of untreated plants and of the controls treated with lanolin at full bloom dried out in much the same manner, except that the changes incident to penetration of the pollen tubes did not occur and a periderm developed across the tissue of the stylar canal.

Following treatment with indoleacetic acid at full bloom, disintegration of the apical tissues was greatly retarded. At the end of 20 days the tissues of the nectary and floral tube were usually shriveled, but cells of the neck were alive. A periderm was not generally formed at the base of the neck, but tangential cell divisions were commonly observed in the peripheral neck region. Cell walls in this zone often became somewhat thickened, with conspicuous pits, but no stone cells or fibers were found. There was evidence of slight proliferation throughout the parenchymatous cells of the neck.

Earlier than 4 days before full bloom meristematic activity was abundant in the tissues of the neck. New cell walls were formed, chiefly in the transverse plane of the axis, so that the cells of the parenchyma presented a more or less tabular or ladder-like appearance. In lanolin controls 4 days before full bloom this pattern was recognizable as long as 24 days later. Except for moderate increase in cell size, the tissues of the neck remained much as they were at the time of treatment. The cells of the nectary and floral tube gradually degenerated. Only slight thickening of cell walls was observed, and no sclerotic or vascular tissues were differentiated subsequent to pre-bloom treatments with pure lanolin paste.

When the 2 per cent acid-lanolin mixture was applied 4 days before full bloom, there was a prompt response in the floral tube, nectary, and neck. Cells immediately below the cut surface of the nectary were slightly enlarged after 24 hours; the nuclei were larger and showed greater affinity for the stains; the cytoplasm increased in amount and was often extended across the large central vacuoles. Apparently normal cell divisions occurred. Continued increase in cell size was chiefly in the direction of the fruit axis, so that markedly elongate cells were formed. By repeated mitotic divisions these cells were divided into groups of smaller cells. The outlines of the parent cell walls were often discernible for several days, much as described in stems of *Lilium* by BEAL (2). In the nectary of the cucumber, however, the new walls were all formed in a single plane, so that in longitudinal section the entire unit of derived cells appeared as a single row. After the initial activity, mitotic divisions gradually decreased and stopped. Cell enlargement continued for about 12 days, at which time the tumors had reached their maximum size. Responses in the floral tube were much less striking than those of the nectary, and there was no evidence of meristematic activity in the stylar canal tissue. Divisions in other than the transverse plane were observed in the neck, particularly in the outer ring of bundles and adjacent parenchyma. Within the bundles cambial activity was initiated and a few parenchymatous derivatives

were formed. In no case were root primordia formed, and no new bundles were differentiated in the proliferated tissue. Meristematic activity ceased after a relatively short period. Thereafter all cells except mature conducting elements of the xylem increased in size, and density of protoplasmic contents decreased throughout. In old tumors the walls of eight or ten peripheral cell layers in the neck region increased considerably in thickness and showed conspicuous pits. Conducting elements of the phloem were no longer recognizable as such, and a few very thick-walled pitted fibers were sometimes found in the outer part of the external phloem.

When the transverse cut was made in such a way that only a portion of the nectary was removed, there was very little activity beneath the uninjured surface; when the cut was above the nectary, proliferation was marked only in the stylar tissues. In this case the style became considerably elongated.

Discussion

Although relatively few species have been investigated, wide variation has been found in the extent of seed-coat development in artificially produced parthenocarpic fruits. In the present investigation there was a progressive decrease from the normal seed-coat size in response to different treatments in the following order: after pollination, the seeds were large; untreated fruits and controls treated with pure lanolin produced slightly smaller abortive seeds; following indoleacetic acid at full bloom development was still further retarded; and the acid applied 4 days before full bloom resulted in the smallest seeds (approximately half the usual size). The small seed coats were only partially hardened. Fruits of the last-mentioned series were decidedly smaller than those which were pollinated. Untreated fruits and controls treated with pure lanolin were as large as those which were pollinated. In the cucumber variety studied, therefore, the presence or absence of an embryo is not a major factor affecting the size of the fruit. Whether the indoleacetic acid has a direct retarding effect on fruit development or acts through changes in the food supply or in some other manner is not known. Chemical analyses of fruits produced by artificial parthenocarpy are needed. Further histological studies to show the relative importance of cell division and cell enlargement to final fruit size would also be desirable.

Following pollination, the nectary, floral tube, and neck of the flower rapidly dry out. The development of an active growing tissue in response to early application of the acid-lanolin mixture from a portion of the flower that would soon shrivel and die in the usual course of events is of interest. Since no true abscission layer is formed at the base of the cucumber neck, this is of different character from the effects of growth substances on abscission of petioles and other organs. In contrast to the results in the bean, the proliferation of tissues of the cucumber fruit is limited to a fairly short period of time, and no vascular derivatives or root primordia are formed.

Summary

1. The development of cucumber fruits of a naturally parthenocarpic variety (Rollison's Telegraph) was studied before and following pollination. The development of megagametophyte, embryo, and seed coats was observed in detail. Tissues of the perianth, stigma, nectary, and neck shriveled within a week after pollination, and a protective periderm formed at the apex of the live tissue

beneath. Twenty days after full bloom hardened seed coats contained large well-developed embryos.

2. Development of untreated fruits in which there was no pollination was essentially the same, except for the absence of embryos and endosperm, the disintegration of megagametophytes, and the slightly smaller size of the seed coats.

3. In other cases transverse cuts were made through the lower portion of the floral tube and a 2 per cent mixture of indoleacetic acid in lanolin was applied to the cut surface.

4. When application of the mixture was made 4 days before full bloom, apical tumors were formed, involving tissues of the nectary, floral tube, and neck. These reached maximum size in about 12 days. Neither vascular tissues nor root primordia were differentiated in the tissues of the tumors. Ovules developed to about half normal size, and seed coats became partially hardened.

5. When pure lanolin was applied 4 days before full bloom, death of the apical tissue was greatly retarded and usually no periderm was formed. Ovules developed as in untreated controls.

6. When indoleacetic acid in lanolin was applied at full bloom the apical tissues remained alive but proliferated very little. Ovules were intermediate in size between those of controls and those of fruits given the pre-bloom treatments with indoleacetic acid in lanolin.

7. When pure lanolin was applied at full bloom, development was essentially the same as that of untreated controls.

8. In the unfertilized ovules of parthenocarpic fruits the nucellus generally developed into a massive tissue with a large central cavity.

9. Meiosis and megagametophyte development were not noticeably affected by any of the treatments.

10. In a single instance following premeiotic treatment with indoleacetic acid there was some proliferation of gametophytic tissue.

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LITERATURE CITED

1. Barber, Kate G., Comparative histology of fruits and seeds of certain species of Cucurbitaceae. Bot. Gaz. 47:263–310. 1909.
2. Beal, J. M., Histological responses of three species of *Lilium* to indoleacetic acid. Bot. Gaz. 99:881–911. 1938.
3. Fickel, J. F., Ueber die anatomie und Entwicklungsgeschichte der Samenschalen einiger Cucurbitaceen. Bot. Zeit. 34:737–744; 753–760; 769–776; 785–792. 1876.
4. Fitting, H., Weitere entwicklungsphysiologische Untersuchungen an Orchideenblüten. Zeitschr. Bot. 2:225–267. 1910.
5. Gardner, F. E., and Kraus, E. J., Histological comparison of fruits developing parthenocarpically and following pollination. Bot. Gaz. 99: 355–376. 1937.
6. Gardner, F. E., and Marth, P. C., Parthenocarpic fruits induced by spraying with growth promoting compounds. Bot. Gaz. 99:184–195. 1937.
7. Gustafson, F. G., Auxin distribution in fruits and its significance in fruit development. Amer. Jour. Bot. 26:189–194. 1939.
8. ———, Induced parthenocarpy. Bot. Gaz. 99: 840–844. 1938.
9. ———, Induction of fruit development by growth promoting chemicals. Proc. Nat. Acad. Sci. 22:628–636. 1936.
10. ———, Parthenocarpy induced by pollen extracts. Amer. Jour. Bot. 24:102–107. 1937.
11. ———, The cause of natural parthenocarpy. Amer. Jour. Bot. 26:135–138. 1939.

12. Hartley, C. P., Injurious effect of premature pollination, with general notes on artificial pollination and the setting of fruits without pollination. U.S. Bur. Plant Ind. Bull. 22. 1902.

13. Kirschner, O., Parthenogenesis bei bluten-pflanzen. Ber. deutsch. Bot. Ges. 22:83–97. 1904.

14. Kraus, E. J., Brown, Nellie A., and Hamner, K. C., Histological reactions of bean plants to indoleacetic acid. Bot. Gaz. 98:370–420. 1936.

15. Noll, F., Über Fruchtbildung ohne vorausgegangene Bestäubung (Parthenokarpie) bei der Gurke. Sitz Ber. der Niederhein. Gesell. Natur. Heilkunde. Bonn. 1902.

16. Tillman, Opal L., The embryo sac and embryo of *Cucumis sativus*. Ohio Naturalist 6:423–430. 1906.

17. Tukey, H. B., Development of cherry and peach fruits as affected by destruction of the embryo. Bot. Gaz. 98:1–24. 1936.

18. Van Overbeek, J., Conklin, M. E., and Blakeslee, A. F., Induction of pseudo-embryos in *Datura* by auxin treatment. Abstract, Amer. Jour. Bot. 27:18s. 1940.

19. Wong, C. Y., A study of chemically induced parthenocarpy in certain horticultural plants, with special reference to the watermelon. Bot. Gaz. 103:64–86. 1941.

20. Yasuda, S., Parthenocarpy caused by the stimulation of pollination in some plants of the Cucurbitaceae. Agr. and Hort. 10:1385–1390. 1935.

EFFECTIVENESS OF GROWTH SUBSTANCES IN DELAYING ABSCISSION OF COLEUS PETIOLES

F. E. GARDNER[1] AND W. C. COOPER[2]

Introduction

An important practical attribute of the plant-hormone chemicals as a class is their ability, in varying degrees of effectiveness, to prevent or delay abscission of flowers, fruits, and leaves. It is recognized that abscission, from an anatomical standpoint, is different in various plant organs, and this fact may influence greatly the response to hormone application. The immediate object of the following experiments was to determine the relative effectiveness of a number of plant hormones in delaying leaf abscission when these chemicals were applied in various ways. In the course of the experiments some responses of physiological interest, incidental to the tests for comparative effectiveness of the compounds, were encountered and are included.

METHODS.—It is a common observation that when leaf blades are removed, leaving all or part of the petioles attached to the stem, the petioles abscise usually within a few days. LA RUE (4), using *Coleus*, demonstrated that indoleacetic acid in a lanolin paste applied to the cut ends of the petioles delayed their abscission. Basically this same procedure has been adopted in these experiments, using the time required for abscission as a measure of the effectiveness of any hormone or concentration of hormone. *Coleus*, the test plant used, has a number of advantages. Any given strain can be easily and quickly increased by vegetative means, and large numbers of uniform, single-stemmed plants can be selected for test purposes. When the leaf blades are removed the untreated petioles begin to abscise after about 48 hours, and the process is largely completed within 3 days, provided the temperature is fairly high. Hormone-treated petioles, on the other hand, may persist for weeks.

For the purpose of these experiments, uniform cuttings were taken from a "mother" bed and rooted in a greenhouse bench. The plants developed rapidly in the bench, and when approximately 8 inches tall, the petioles of the top two pairs of fully expanded leaves of each plant were used. Petioles of immature leaves were found to persist too long—even without hormone treatment—to be used for accurate test purposes. For each hormone treatment twelve plants involving forty-eight petioles were employed. In many cases twenty-four instead of twelve plants were used and the treatments replicated, so that half the plants occurred at two different locations in the bench, in order to disclose any positional effects. Although considerable differences in growth were evident in various portions of the bench, no very marked differences in time of petiole abscission within a given treatment were disclosed.

Two methods of applying each of the hormones were used. In the first method the hormones in a 5 per cent ethyl alcohol solution[3] were sprayed on the intact

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[3] Because of the relatively high concentrations of growth substances used, alcohol up to 5 per cent in the aqueous solution was employed to increase solubility. A 5 per cent alcohol solution was in itself without visible effect on the plants.

plants, and 2 days later the leaf blades were cut from the selected petioles. Abscission records were dated from the day the blades were removed. In the second method the blades were cut off with a sharp knife, and a drop of the hormone-lanolin mixture, liquefied by warming, was applied to cover the cut surface of each petiole. In this procedure two lengths of petiole, 0.5 cm. and 2 cm., were allowed to remain on the plant. Some interesting responses, involving transport of effect, were disclosed by using these different petiole lengths. Figure 1 shows individual plants with four leaf blades removed, leaving the petioles 0.5 and 2 cm., respectively, ready for application of the growth substance.

Rate of abscission, as well as effectiveness of the compounds, is unquestionably affected by temperature. With relatively low temperatures the time of normal abscission is delayed and also the effectiveness of the compounds reduced. In these experiments no special efforts were made to control the temperature of the greenhouse, but all comparable treatments were included within the same series and so were subject to the same fluctuations of temperature, light, and humidity. When one series, or bench of plants, was completed it was discarded and new plants prepared for the following series. Throughout the experiments the same strain of *Coleus* was employed.

Counts of petioles remaining on the plants after treatment were made daily or every other day, depending on the abscission rate. In making these counts the petioles were lightly touched to dislodge any which might still be attached although abscission had taken place. The various compounds were tested at four concentrations, 0.01, 0.02, 0.05, and 0.1 per cent, both as water-alcohol sprays and in lanolin. The following compounds were included in careful comparative tests:

Indole compounds	Naphthalene compounds
β-Indoleacetic acid	α-Naphthaleneacetic acid
γ-Indolebutyric acid	β-Naphthoxyacetic acid
Methyl indolebutyrate	α-Naphthylmethylacetate
β-Indole acetamide	α-Naphthyl acetamide
	α-Naphthyl thioacetamide

In addition to these recognized plant-growth substances, a large number of

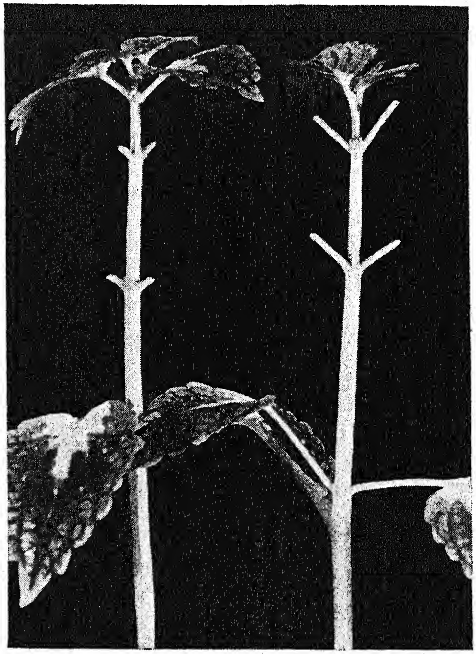


FIG. 1.—*Coleus* plants with petioles cut to 0.5 and 2 cm., ready for application of lanolin-growth substance mixtures.

miscellaneous compounds were tested for activity in delaying abscission by applying them in lanolin at 1 per cent concentration to the petiole stubs.

In preliminary tests it was found that the effect of naphthalene acetonitrile and of the propionic and butyric acids of naphthalene was so slight in delaying petiole drop that they were omitted from

further comparative tests. Likewise no effect could be demonstrated from α-naphthoxyacetic acid, although the β derivative was quite potent.

Results

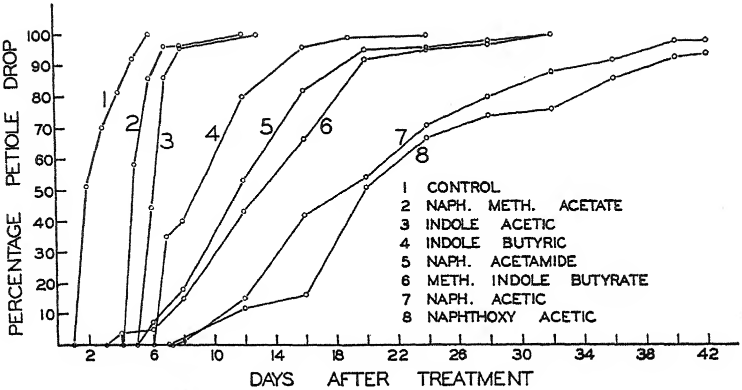
SPRAY APPLICATIONS.—At 0.01 per cent concentration most of the compounds had but slight effect in delaying abscission. β-naphthoxyacetic acid stood out pre-eminently more effective at this concentration than any other compound tested. The maximum effectiveness of all compounds was reached at 0.05 per cent, with the exception of β-indoleacetic acid, which showed still greater effectiveness at 0.1 per cent.

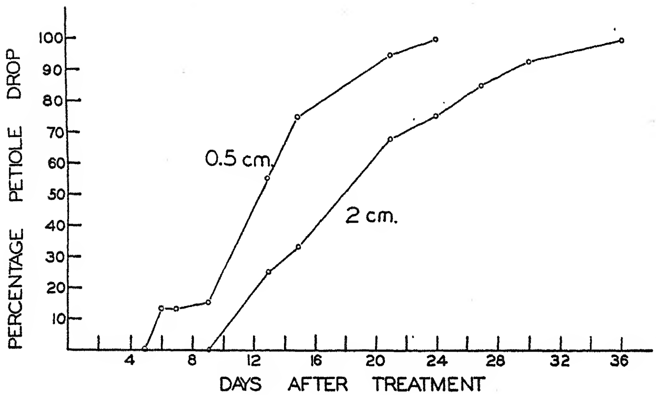
The increased effectiveness of all compounds, with the single exception of indoleacetic acid, with increasing concentrations up to 0.05 per cent but no greater effect at 0.1 per cent was very striking. The limitation of effectiveness of so many compounds at 0.05 per cent suggests that the limiting factor lies within the plant itself, restricting the utilization of more than a definite quantity of hormone. It is not known why indoleacetic acid, the only compound of the lot known to occur in plant tissue, was not similarly restricted, although the fact seems to be without particular significance at present.

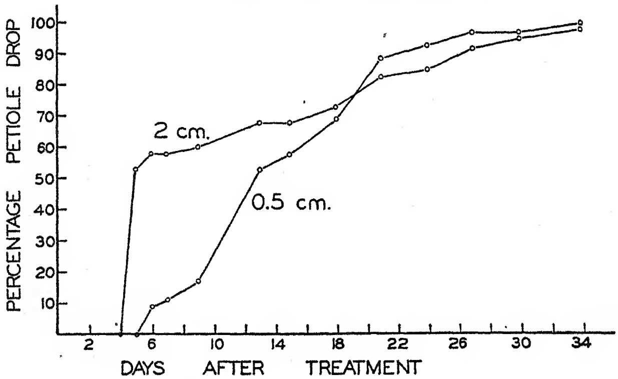
Inasmuch as 0.05 per cent represented the approximate maximum effective concentration of each of the compounds, with the exception of indoleacetic acid already noted, the effect of the various compounds on abscission at 0.05 per cent is shown in figure 2 and represents their maximum relative effectiveness. Omitted from figure 2, to avoid crowding the chart, are the results with (*a*) α-naphthyl thioacetamide, which were nearly identical with naphthyl acetamide itself, and (*b*) indole acetamide, which showed practically no delay in abscission over the control, even when used at 1 per cent spray concentration. The comparison in figure 2 shows β-naphthoxyacetic acid to be the most effective compound tested, followed in order by α-naphthaleneacetic acid and methyl indolebutyrate. It will be noted presently that the order of relative effectiveness was quite different when the same compounds were applied by the lanolin method.

LANOLIN APPLICATIONS.—Before attempting to present the evidence on the relative effectiveness of the various compounds in lanolin applications, the factors which complicate this presentation should first be considered. The chief was the finding that the length of petiole through which the hormone must act to affect the point of abscission in large measure determined the effectiveness of a given compound. This in itself would not be any particular complication were it not for the fact that the effect of all indole compounds was increased by acting through long petioles, whereas the effect of all naphthalene compounds was thereby apparently decreased, owing to a lag in time before the naphthalene compounds became effective. Figures 3 and 4 show the abscission rates with a typical compound of each class with petiole lengths at 0.5 and 2 cm.

The time lag before naphthyl thioacetamide took effect in the case of the 2-cm. petioles permitted more than 50 per cent of the petioles to fall (fig. 4). After starting to react, however, this compound appeared to be more effective on 2-cm. petioles, as evidenced by the slope of the curve, than in the case of those 0.5 cm. long. The conclusion is probably justified that increased petiole length enhances the actual effectiveness of both indole and naphthalene compounds, but







FIGS. 2–4.—Fig. 2 (top), comparative effectiveness of various growth substances applied as sprays at 0.05 per cent concentration, in delaying petiole abscission. Fig. 3 (center), delay of abscission with indoleacetic acid, applied in lanolin at 0.1 per cent concentration, showing greater effectiveness by acting through the longer petioles; this was typical of all indole compounds. Fig. 4 (bottom), delay of abscission with naphthalene thioacetamide, applied in lanolin at 0.1 per cent concentration, showing lag in start of effectiveness with the longer petioles; this characterized the action of all naphthalene compounds.

that, owing to the lag in the case of the naphthalene compounds, increased length of petiole decreased the practical effectiveness of this latter group. The lag in the case of naphthyl thioacetamide was somewhat more pronounced than with most of the other naphthalene compounds and was therefore purposely selected to demonstrate the point.

tween control petioles 2 cm. long and those 0.5 cm. long.

It is apparent from table 1 that, as in the case of the spray applications, the 0.05 per cent mixture in general marked the maximum effective concentration. There were minor exceptions to this rule, one of which was indoleacetic acid, which has consistently showed increased activity above the 0.05 per cent concentration. All the indole compounds showed greater activity with 2-cm. petioles than with those 0.5 cm. The reverse situation was true of the naphthalene compounds, reflecting the situation already described with respect to the time lag when naphthalene compounds were used on the longer petioles.

TABLE 1

RELATIVE EFFECTIVENESS OF VARIOUS HORMONES IN PREVENTING ABSCISSION WHEN APPLIED IN LANOLIN TO CUT PETIOLE ENDS

COMPOUND	DAYS REQUIRED FOR 50 PER CENT OF PETIOLES TO DROP					
	0.1% conc.		0.05% conc.		0.01% conc.	
	0.5 cm.	2 cm.	0.5 cm.	2 cm.	0.5 cm.	2 cm.
Indole group						
Methyl indolebutyrate	24	29	22	25	15	18
Indolebutyric acid*	14	16	14	16	10	12
Indoleacetic acid	13	18	11	15	10	12
Indole acetamide	11	13	11	13	5	9
Naphthalene group						
α-Naphthaleneacetic acid	24	12	19	13	5	5
β-Naphthoxyacetic acid	13	10	13	8	12	9
α-Naphthylmethylacetate	11	10	11	9	9	4
α-Naphthyl acetamide	12	11	12	9	3	2
α-Naphthyl thioacetamide	11	5	11	4	2	2

*In a subsequent test the 0.1 per cent concentration of indolebutyric showed marked increased effectiveness over the 0.05 per cent. The temperature was unusually high during this latter test but is not offered as an explanation of the seeming inconsistency. It is probable that the relative effectiveness of this compound is higher than here indicated.

Any comparison of the relative practical effectiveness of the various indole and naphthalene compounds tested by the lanolin method would obviously have to take into consideration the matter of petiole length. Table 1 shows the time in days required for 50 per cent of the petioles to abscise for each of the compounds used at three different concentrations on petioles of 0.5 and 2 cm. lengths. With the pure lanolin control plants, more than 50 per cent of the petioles dropped in 2 days, and no significant difference in time of dropping could be detected be-

Considering all three concentrations (table 1), it is believed that the order of the compounds as given in the table represents their relative effectiveness within the indole and naphthalene groups. This

order, of course, applies to lanolin applications of the hormones to the cut ends of the petioles and differs from the order shown in figure 2 for spray applications of the same compounds.

Miscellaneous compounds.—Since a great number of miscellaneous organic compounds and a simple test method in the *Coleus* technique were available, it was decided to make at least a preliminary test of the entire group of chemicals for activity in delaying abscission. In many instances there were reasons, based on molecular configuration, for anticipating that the compounds did not possess hormone activity, but they were nevertheless employed. All the following compounds were tested at 1 per cent concentration in lanolin applied to 1-cm. petiole stubs, as previously described. Even at this relatively high concentration most of the compounds failed to produce any effect on abscission of the *Coleus* petioles. A few produced such slight effect that it would require more careful testing to establish it definitely, and even then the degree of such activity would be so slight as to preclude any practical advantage from their use. The list is presented only as a matter of record.

Compounds tested

1. Acetoacetanilide
2. Acetoacet-2-chloroanilide
3. Acetoacet-2, 5-dichloroanilide
4. Acetophenone
5. Acetyl-o-aminobenzoic acid
6. Acetyl-o-anisidine
7. Acetyl-p-anisidine
8. Acetyl diphenylamine
9. Acetyl-o-phenetidine
10. Acetyl-p-phenetidine
11. Acetyl phenylhydrazine
12. Acridone
13. 1-Aminoanthraquinone
14. 2-Aminoanthraquinone
15. 2-Amino-5-azoanisole
16. p-Aminoazobenzene
17. 2-Amino-5-azotoluene
18. p-Aminobenzophenone
19. p-Aminodiphenyl
20. 2-Amino-4-nitrophenol
21. o-Aminophenol
22. p-tert.-Amylphenol
23. Anethole
24. Aniline
25. Anisaldehyde
26. Anisic acid
27. p-Anisidine
28. Anisalacetone
29. Anthracene
30. Anthragallol
31. Anthranilic acid
32. Methylanthranilic acid
33. Methyl anthranilate
34. Azoxybenzene
35. Barbituric acid
36. Benzalacetone
37. p-Benzalaminophenol
38. Benzalaniline
39. Benzaldehyde phenylhydrazone
40. Benzamide
41. Benzanilide
42. Benzenoazo-o-cresol
43. Benzene hexachloride
44. Benzeneazoresorcinol
45. Benzil
46. Benzilic acid
47. Benzoic anhydride
48. Benzoin
49. Benzoyl-o-toluidine
50. Benzylaniline
51. o-Benzylphenol
52. p-Benzylphenol
53. Benzylphenylnitrosoamine
54. Benzyl sulfide
55. β'-Butoxy-β-ethoxyethyl acetate
56. p-Bromoacetanilide
57. p-Bromoanisole
58. o-Bromoanisole
59. Bromobenzene
60. o-Bromobenzoic acid
61. m-Bromobenzoic acid
62. p-Bromobenzoic acid
63. p-Bromodiphenyl
64. p-Bromophenetole
65. o-Bromophenetole
66. n-Butylbenzene
67. sec.-Butylbenzene
68. tert.-Butylbenzene
69. n-Butyranilide
70. n-Caproanilide
71. Carbazole
72. Carvone
73. Chloranil
74. p-Chloroacetanilide
75. β-Chloroanthraquinone
76. o-Chlorobenzoic acid
77. p-Chlorobenzoic acid
78. o-Chlorodiphenyl
79. Chlorohydroquinone
80. p-Chlorophenetole

81. o-Chlorophenol
82. 2-Chloro-o-phenylphenol
83. 4-Chloro-o-phenylphenol
84. p-Chlorothymol
85. m-Chlorotoluene
86. Chrysene
87. Cinchonidine
88. Cinchonidine salicylate
89. Cinnamic acid
90. Cresidine
91. m-Cresol
92. p-Cresol
93. m-Cresyl benzoate
94. p-Cresyl benzoate
95. p-Cyclohexylanisole
96. o-Cyclohexylphenol
97. p-Cyclohexylphenol
98. Dextrose
99. Diacetyl benzidine
100. 2, 4-Diaminodiphenylamine
101. 2, 4-Diaminotoluene
102. Dianisalacetone
103. Dianisidine
104. Dibenzalacetone
105. p-Dibenzoidioxin
106. Dibenzylaniline
107. p-Dibromobenzene
108. 2, 5-Dichloroaniline
109. p, p′-Dichloroazoxybenzene
110. 4, 4′, Dichloro-2, 2′-dinitrodiphenyl
111. 4, 4′, Dichloro-2, 2′-dinitrodiphenyl sulfide
112. 4, 4′-Dichlorodiphenyl
113. 4, 6-Dibromo-o-cresol
114. 2, 6-Dichloro-4-nitroaniline
115. 1, 5-Dihydroxyanthraquinone
116. 2, 4-Dihydroxyazobenzene-4′-sulfonic acid
117. 1, 3-Dihydroxy-4-chlorobenzene
118. Ethylene glycol diacetate
119. Furoic acid
120. Methyl furoate
121. n-Propyl furoate
122. n-Butyl furoate
123. n-Isoamyl furoate
124. Furfural
125. Furfuryl alcohol
126. Furfuryl acetate
127. Furfuryl diacetate
128. Hydrofuramide
129. α-Methyl-α-phenylhydrazine
130. Salicylacetic acid
131. Sulfanilic acid
132. Phenothiazine
133. N-Phenylanthranilic acid
134. Phenylacetic acid
135. Potassium-n-butylxanthate
136. Uric acid

Naphthalene compounds

137. 2, 4-Dibromo-1-naphthol
138. 2, 4-Dichloro-1-naphthol
139. α-Naphthyl acetate
140. β-Naphthyl acetate
141. β-Naphthyl benzoate
142. β-Naphthoylacetonitrile
143. Sodium-1-naphthol-4-sulfonate
144. Naphthalic acid
145. 2-Aceto-1-naphthol
146. Acenaphthene
147. α-Naphthylcarbinol
148. Acenaphthoyl-3-propionic acid
149. 1-Methyl naphthyl-4-methyl thiocyanate
150. 1-Methyl naphthyl-4-methyl isothiocyanate
151. 2-Methyl naphthyl-1-methyl thiocyanate
152. 2-Methyl naphthyl-1-methyl isothiocyanate
153. Tetralyl-6-methyl thiocyanate
154. Tetralyl-6-methyl isothiocyanate
155. α-Naphthyl methyl thiocyanate
156. α-Naphthyl methyl isothiocyanate

Discussion

In trying to anticipate which of the available growth substances to apply to correct any given abscission problem, and also what range of concentrations to employ, a number of factors should be considered. Perhaps the first of these is an anatomical consideration of the type of abscission involved. At least two general types are recognized: (*a*) that characterized by the formation of a definite pre-formed abscission layer resulting from secondary cell division such as usually precedes the dropping of flowers, leaves, and young fruits; and (*b*) that characterized chiefly by weakening changes in the cell walls in an abscission zone, as has been reported by MacDaniels (5) and McCown (6) preceding the drop of mature apple fruits. Delay of abscission by growth substances in one instance retards cell division, whereas in the other instance cell-wall weakening may be interrupted. The distinction between the two types of abscission is not a hard and fast one, for in the type characterized chiefly by cell-wall changes some cell division is also found, and in the first type cell-wall changes in the newly formed abscission layer subsequently take place before actual abscission occurs. Myers (7), in a study of abscission

in *Coleus* leaves, described these changes. He also reported that the application of growth substances to debladed *Coleus* petioles retarded the initiation of the abscission layer and its growth at any stage of development. The growth substance most effective in one type of abscission is not necessarily most effective in the other. Unfortunately, evidence is not available on a sufficient number of plant species to support a statement as to which growth substances are most effective in delaying each type of abscission. For example, the abundant evidence on the effect of the various growth substances in the rooting of cuttings shows that the different species and varieties differ considerably in their relative response to the various substances. As to range of concentrations to be employed, the limited evidence available suggests that the low concentrations, in the neighborhood of 0.0005 per cent, found to be very effective against abscission of the second type, as in the preharvest drop of apples (2), are much too dilute to be of value in the first type, of which leaf abscission is an example.

Another factor, although probably over-emphasized in the consideration of the choice of growth substance for a particular purpose, is its relative solubility in the carrier to be used. For practical purposes it is probable that a relatively low solubility, within reasonable limits, resulting in an incomplete solution, does not necessarily impair the effectiveness of a compound but may, in fact, enhance it by providing a film of finely divided particles on the surface of the plant which may gain entrance over an extended period. The excellent results obtained by HOFFMAN (3) in controlling apple drop by hormone dusts are evidence of this, as are also the results with lanolin-emulsion sprays for various purposes and the use of hormone dusts for rooting of cuttings. It should be recorded that in these tests it was not possible with the spray applications to effect complete solution of all the compounds at the higher concentrations, even with 5 per cent alcohol which was used as a carrier. All compounds were in complete solution at the 0.01 per cent concentration, but at 0.05 and 0.1 per cent, methyl indolebutyrate, naphthyl methylacetate, naphthyl acetamide, and thioacetamide were incompletely dissolved. As suspensions or emulsions they were delivered to the plant surface in the same absolute amounts as the other compounds completely in solution. The lanolin mixtures also may not have been complete solutions, for, even though heated and the compounds apparently dissolved in the melted lanolin, it was not possible to be sure that, when cooled, the solubility was not exceeded. It is, of course, not possible to say whether the relative results with the various compounds would have been different had they all been completely dissolved. For the practical considerations involved it makes little difference, inasmuch as the results of any comparative tests of these compounds must of necessity be qualified by the solubility of the compounds and by many other unknown characteristics, such as the relative rate of entrance into the plant tissue, their rate of movement in the tissue, and their relative inactivation or destruction by enzymes or other compounds within the plant.

Entirely apart from its effectiveness for controlling abscission, another factor to be considered in the selection of a growth substance is the avoidance of certain undesirable deforming effects. β-naphthoxyacetic, indolebutyric, and α-naphthaleneacetic acids are especially prone to result in aerial rooting and

marked distortion by bending and twisting of young foliage and shoots. Certain "formative effects" with β-naphthoxyacetic acid and certain other compounds have been noted (10, 11). In general, these undesirable side effects are severe only on immature or succulent growth and with relatively high concentrations of the compounds. On the other hand, naphthalene acetamide is practically free of these effects.

Two interesting features have been disclosed in this study which may eventually prove important in helping to understand the nature of the hormone action in preventing abscission and in the practical application of these substances to counteract undesirable dropping of plant organs. These two features are the delay in effect of the naphthalene compounds when applied to the petiole some distance from the point of abscission and the increased effectiveness of the indole compounds—and probably also of the naphthalene compounds, once their action begins—by acting through the longer lengths of petiole.

The time lag in the action of the naphthalene compounds through long petioles can probably be ascribed to a slower rate of longitudinal transport of this group of compounds than is characteristic of the indole compounds. No suggestion is advanced as to why this should be true. WENT and WHITE (9) present evidence which indicates that naphthaleneacetic acid is transported longitudinally through *Avena* coleoptiles at a much slower rate than either of two indole compounds also tested. From the standpoint of practical application of the naphthalene compounds this slower rate of transport has significance only when the growth substance must be applied some distance from the point of desired activity. With short petioles, where the distance between point of application and point of abscission is small, or with spray applications in which the compound reaches the abscission point directly, the lag was obviated.

The increased effectiveness by acting through the longer petioles in the case of the indole compounds, and of the naphthalene compounds as well—except for their lag—suggests that the action of the growth substances depends on or is conditioned by the presence of a necessary second substance or substances already present in the plant tissue. Obviously there should be more of the second substance or substances in a long petiole than in a short one, and the amount present may be a factor limiting the action of the growth substance itself. The maximum effective concentration of so many of the growth substances in this study at approximately 0.05 per cent lends further support to the presence of naturally occurring factors within the plant which limit the effectiveness of applied growth substances. It is not known, however, whether the naturally occurring factor or factors are simply metabolic substances such as carbohydrates and proteins, which are undoubtedly essential to all activity by the plant and have been shown by various workers to influence the response to applied growth substances, or whether these factors can be classified as specific hormone-like substances designated as "calines" by WENT (8). EYSTER (1) has recently proposed that the action of "auxins" is due fundamentally to the release of diastase and possibly other enzymes from the protein colloids to which they are normally attached.

Summary

1. The relative effectiveness of a number of growth substances, both indole and

naphthalene derivatives, in delaying leaf abscission in *Coleus* was tested by two methods of applying the compounds: by aqueous sprays and by lanolin mixtures applied by hand to cut petiole surfaces.

2. In the case of the spray applications the maximum effectiveness in delaying abscission was reached at approximately 0.05 per cent concentration of all compounds, with the exception of indoleacetic acid, which showed still greater effect at 0.1 per cent concentration. In most instances with the lanolin applications 0.05 per cent also represented the approximate maximum effective concentration.

3. The relative order of effectiveness of the various compounds differed considerably in the two methods of application. At the maximum effective concentration methyl indolebutyrate was the most effective in lanolin applications on petiole stubs, whereas in the spray application method β-naphthoxyacetic acid proved most effective.

4. With all napthalene compounds applied by the lanolin method there was an appreciable lag between time of application and the beginning of effect in preventing abscission, while the indole compounds took effect promptly. The results suggested that the naphthalene compounds were transmitted longitudinally through the petioles at a slower rate than the indole compounds.

5. The indole compounds, and also the naphthalene compounds, once their action was initiated, exhibited greater effectiveness on petioles 2 cm. long than on 0.5 cm. lengths. This suggested the possibility of the presence in the petioles of a naturally occurring second substance or substances necessary for the action of the applied growth substance, although it does not imply that such substances are specific hormone-like factors.

6. The prevention of leaf abscission appears to require a much higher concentration of applied growth substance than the prevention of mature fruit drop. This may be related to the differences in the types of abscission involved.

7. Of 156 additional miscellaneous organic compounds tested for activity in delaying petiole drop, none showed sufficient activity for practical usage.

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LITERATURE CITED

1. Eyster, H. C., Auxin action Science 97:358–359. 1943.
2. Gardner, F. E., and Marth, P. C., Spraying with plant growth substances for control of the pre-harvest drop of apples. Proc. Amer. Soc. Hort. Sci. 37:415–428. 1939.
3. Hoffman, M. B., Edgerton, L. J., and Van Doren, A., Some results in controlling the preharvest drop of apples. Proc. Amer. Soc. Hort. Sci. 40:35–38. 1940.
4. La Rue, C. D., The effect of auxin on the abscission of petioles. Proc. Nat. Acad. Sci. 22: 254–259. 1936.
5. MacDaniels, L. H., Some anatomical aspects of apple flower and fruit abscission. Proc. Amer. Soc. Hort. Sci. 34:122–219. 1936.
6. McCown, M., Abscission of flowers and fruits of the apple. Proc. Amer. Soc. Hort. Sci. 36: 320. 1939.
7. Myers, R. M., Effect of growth substances on the absciss layer in leaves of *Coleus*. Bot. Gaz. 102:323–337. 1940.
8. Went, F. W., Specific factors other than auxin affecting growth and root formation. Plant Physiol. 13:55–80. 1938.
9. Went, F. W., and White, Ralph, Experiments on the transport of auxin. Bot. Gaz. 100:465–484. 1939.
10. Zimmerman, P. W., and Hitchcock, A. E., Formative effects induced with β-naphthoxyacetic acid. Contrib. Boyce Thompson Inst. 12:1–14. 1941.
11. ———, and ———, Flowering habit and correlation of organs modified by triiodobenzoic acid. Contrib. Boyce Thompson Inst. 12:491–496. 1942.

DEVELOPMENT OF THE FIBROUS NET IN THE FRUIT OF VARIOUS RACES OF LUFFA CYLINDRICA

EDMUND W. SINNOTT AND ROBERT BLOCH

Introduction

The fibrous network of the fruit of *Luffa cylindrica*, the "vegetable sponge" or "sponge gourd," is of considerable economic importance, since it is used for a wide variety of purposes, from bath sponges to oil filters in marine engines. The literature on these plants does not include any account of the development and histology of the fibrous net or a comparison of its character in the various types. This paper presents the results of such a developmental analysis on a number of diverse races in this species.

Seeds of *Luffa* were obtained from various commercial sources and from the Department of Agriculture; they originated from several warm-temperate regions, notably Japan, Afghanistan, and various parts of the United States. About thirty distinct races were grown. All were planted outdoors in the Marsh Botanical Garden at New Haven, Connecticut, early in May, 1942. Some of the most promising lines were also started in the greenhouse and set out in the field when danger from frost was past. These for some time maintained their size advantage over the ones planted outdoors. Many of the races grew poorly and were obviously unsuited to our climate, but a considerable number successfully produced mature fruits. It is only these latter which were intensively studied.

In addition to *L. cylindrica*, which is the chief source of the commercial product, a number of races of *L. acutangula* were also grown. Only a brief report of these will be made in the present paper.

METHODS.—The plants, well fertilized, were grown on a trellis and were planted 9 feet apart each way. No serious trouble from insect or fungous pests was encountered nor were any of the plants affected by mosaic. The season was an unusually good one, and there was no killing frost until November.

The races of *L. cylindrica* chiefly studied included several of the long narrow type, with a length of about 1200 cm. and a width of 70 cm.; others somewhat shorter and wider (600 × 100 cm.); and others considerably smaller (fig. 1). For the various types, self pollinations were successfully made and abundant seed obtained.

By the use of various substances, especially indoleacetic and naphthaleneacetic acids, parthenocarpic fruits were produced. This was accomplished most readily by applying the substance in lanolin paste directly to the stigma on the day preceding the opening of the flowers.

Polyploid plants were produced by treatment of seedlings with a 0.5 per cent solution of colchicine, but they failed to grow vigorously and none produced fruit.

Ovaries were collected at all stages, from a diameter of 1 mm. or less in width to mature fruit. In larger ovaries samples from various regions were taken. Material was killed in Craf, run through butyl-alcohol, imbedded in paraffin, and stained with safranin and fast green. In later stages of net formation, entire young fruits or parts of them were dissected and studied directly by hand sec-

tions and by maceration. In each race, entire mature fruits were harvested and dried and the leathery outer pericarp removed, exposing the entire network. By removing the seeds and retting in water, the fibrous net, free from other structures, was isolated.

Maceration of the mature strands into their component fiber cells proved difficult but was accomplished by prolonged boiling in concentrated potassium hydroxide. Details of the fibers could be brought out well by staining in Ruthenium red or safranin. The relationship of the fibers on the surface of the strand could be determined by staining the entire strand with Ruthenium red and studying under low power.

Results

The ovary is divided into four rather well-marked regions (fig. 2). The central, ovule-bearing portion may be termed the placental region (*D*). The morphological nature of the rest of the ovary is not well understood, but for convenience it may be termed the "wall." Running through it is a series of large vascular bundles, setting off the outer wall (*A*) from the inner portion. In the latter, two definite regions may be distinguished, the inner wall in which the strands of the fiber run

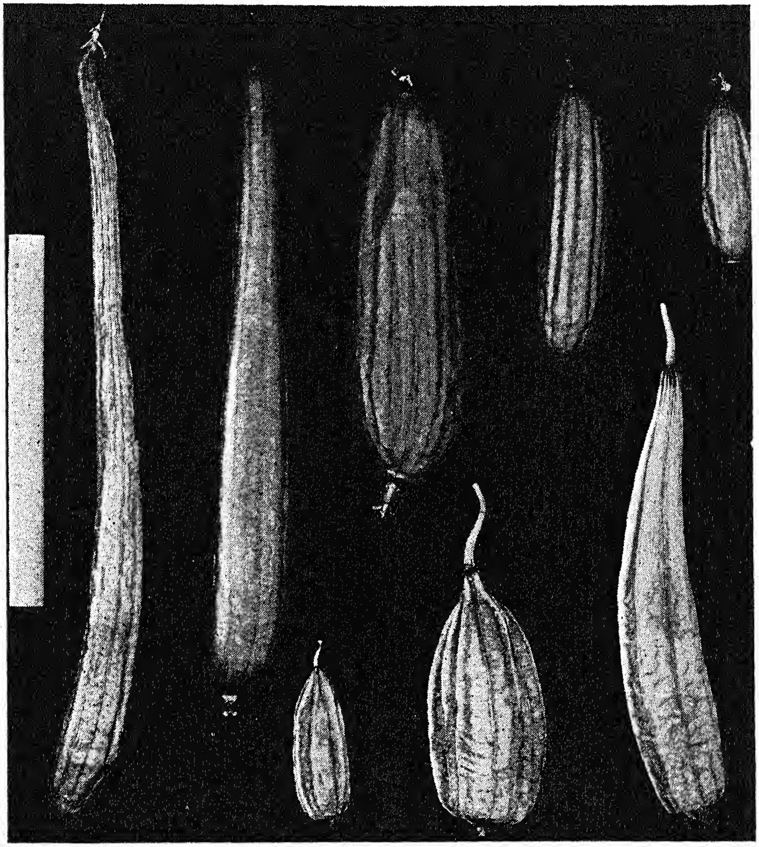


FIG. 1.—*Luffa* fruits: upper row, *L. cylindrica;* lower row, *L. acutangula*

in a chiefly longitudinal direction (*C*) and the middle wall where they run parallel to the transverse circumference (*B*). In the very smallest ovaries the provascular cells of the main vascular bundles, running lengthwise, begin to appear; but when the ovary reaches a diameter of about 1 mm., differentiation of the fibrous network begins. This occurs both in the placental region and in the wall by

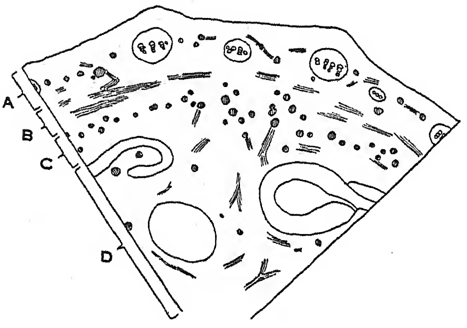


FIG. 2.—Diagram of cross sector of young ovary of *Luffa*. *A*, outer wall showing main longitudinal vascular bundles and small anastomosing fiber strands; *B*, middle wall showing outer transverse strands of fibrous network (outer shell separates from net along line *AB*); *C*, inner wall showing longitudinal fiber strands; *D*, placental region showing ovules and anastomosing fiber strands.

a change in the character of the otherwise uniform parenchymatous cells.

The first visible indication of the future position of a strand is the increased protoplasmic content of a continuous series of parenchyma cells (fig. 5). Each of these soon begins to divide parallel to the axis of the strand. The strand is laid down as a unit simultaneously throughout its course and differentiation does not proceed from cell to cell. Whatever determines the pattern of the future net seems to affect the entire structure and modifies the physiological condition of a series of cells connected with one another in a closed system.

The entire net is not laid down at once, but new strands continue to be differentiated from essentially isodiametric parenchyma cells until some time after fertilization, and often until the fruit is nearly half grown. Thus there is a continuous increase in the complexity of the net pattern as the fruit increases in size. Bundles in the inner wall appear somewhat later than the rest, for this part of the wall develops later. The cessation of strand differentiation in a given region directly follows the cessation of cell division in the parenchymatous cells of the ground tissue in this region. *Luffa* generally agrees with the other cucurbits (5) in which cell division ceases first in the placental region, next in the inner wall, and then in the outer wall. Thus the differentiation of new strands ceases first in the central portion of the fruit and successively in the outer regions.

The strands of the net are not distributed at random but form a very definite pattern which is fundamentally alike in all types but shows certain specific differences in the various races. Around each ovule or group of ovules there is a series of strands, and the mature seed is thus held in a fibrous pocket with an opening on its axial side through which the seed ultimately falls and then drops out of a pore at the tip as the dried fruit is shaken. Surrounding the placental region in the innermost portion of the wall is a series of strands developing comparatively late, often stouter than the rest, most of which run parallel or nearly parallel to the long dimension of the fruit. Outside these is another series of strands which tend to run at right angles to the axis and thus transversely around the outside of the net. In the more delicate fruits there may be only one layer of strands in each set, but in the larger ones there may be many strands and a much more massive system. No bundles pass

upward from the stalk directly into this net, but the vascular supply of the net is entirely through connection with the main vascular bundles in the outer wall, which are continuous with the bundles of the stalk and stem.

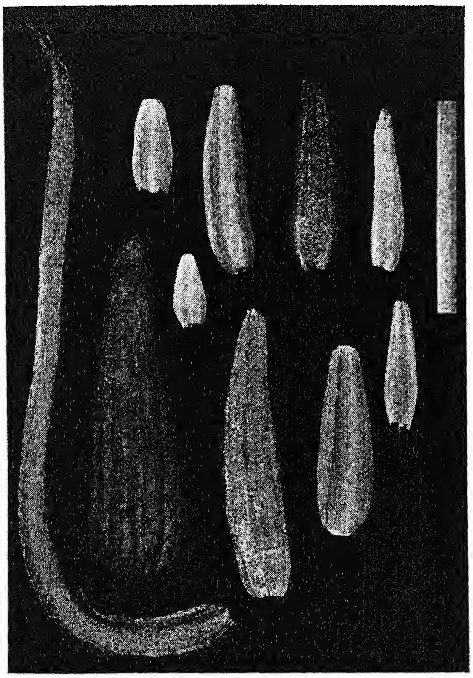


FIG. 3.—Fibrous nets of some of the races studied, showing differences in color, form, and texture.

No strand of the net extends for more than a few millimeters without branching, so that the strands anastomose freely and the entire fibrous system is a closely intercommunicating reticulum. Its details vary considerably in the different races. Some of the more important differences, both as to the whole net and as to its details, are shown in figures 3 and 4.

At maturity the outer wall, most of the cells of which are now sclerotic, becomes a tough dry shell which separates readily from the fibrous net within. This shell contains the main vascular bundles, which are continuous with the vascular system of the stem. Connecting with them and ramifying through this outer wall are many small strands which are continuous with those of the inner net.

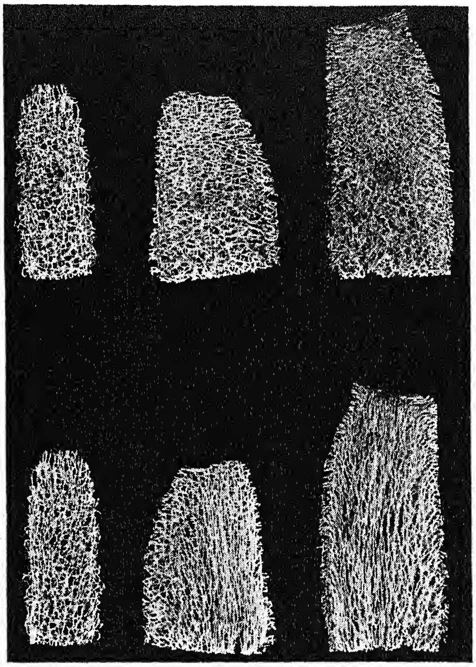
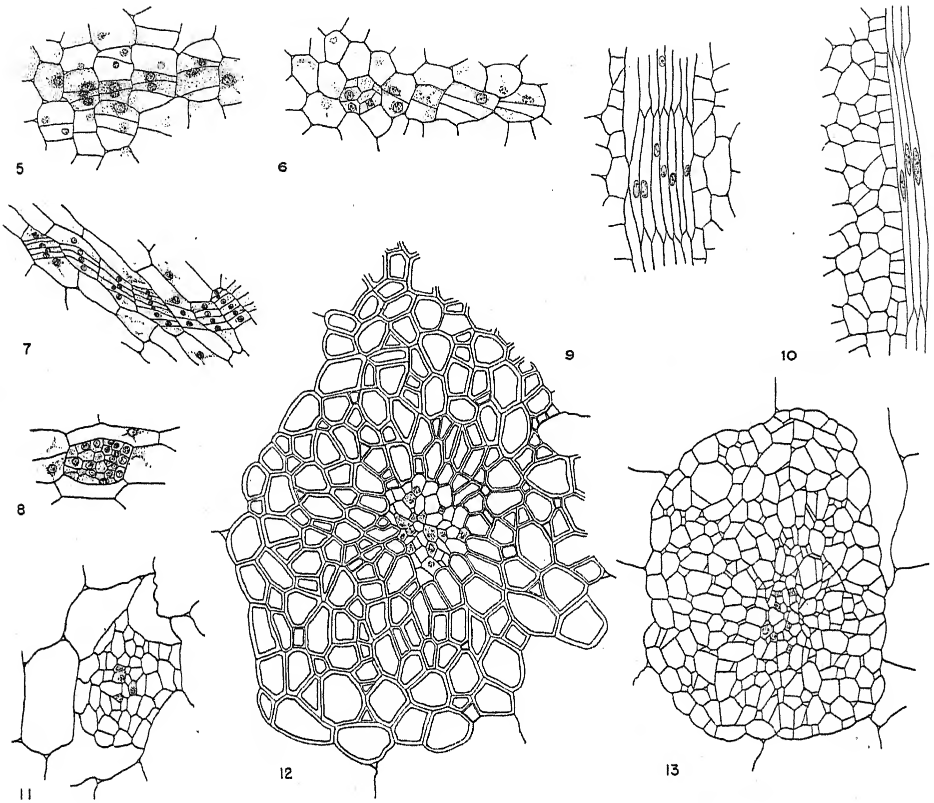


FIG. 4.—Pieces of wall of net from corresponding regions in three races. Above, as seen from fruit surface; below, as seen from inside. Outer series of bundles prevailingly transverse in direction, the inner prevailingly longitudinal. Races differ markedly in radial thickness of fiber system, compactness of network, and diameter of strands.

When the mature wall separates from the net these connections are broken.

In *L. acutangula* there is no sharp line between the sclerotic and the thin-walled parenchyma in the outer layers of the fruit, the former gradually merging into the latter. It is therefore impossible to separate easily the tough outer wall from the fibrous net within, so that this species is much less satisfactory for the production of "sponges" than is *L. cylindrica*.



FIGS. 5–13.—Fig. 5, early stage in formation of a transverse fiber strand, from transverse section through ovary 1.5 mm. wide. ×200. Fig. 6, early stage of strand formation showing (at left) cross section of strand mother cell divided into nine cells and (at right) longisection of young branch strand. ×200. Fig. 7, longisection through slightly older transverse strand showing fiber initials in ground parenchyma cells. Note absence of relation between course of strand and shape of mother cells. ×200. Fig. 8, transection through inner longitudinal strand, somewhat older than that in fig. 6. ×200. Fig. 9, more advanced stage of fiber development with end walls pointed; ovary diameter 10 mm. Note group of fibers which have come from same mother cell. ×200. Fig. 10, still more advanced stage of fiber development showing fibers from only one side of strand; ovary diameter 26 mm. Single group of fibers can still be distinguished. ×115. Fig. 11, transection through young fiber strand of small size with phloem elements in center, from placental region in Line A3. Ovary diameter 8 mm. ×225. Fig. 12, transection through comparable mature fiber strand in same region and line as in fig. 11. Ovary diameter 75 mm. ×225. Fig. 13, transection through fully grown but not yet lignified fiber strand of medium size, showing phloem elements in center and numerous small lumina with acute wall angles, presumably resulting from intrusive growth and branching of fibers. Line D2. Ovary diameter 110 mm. ×115.

The histology of the individual fiber strands varies considerably in different parts of the fruit and among different races. In the rows of densely protoplasmic cells which are the first indication of the position of a strand, the cells begin to divide parallel to the axis of the future strand (figs. 5, 6). This direction bears no necessary relation to the particular shape of the cell, since the strand may twist and turn rather irregularly through the mass of parenchyma. Each cell is cut by these divisions into a considerable number of small elongate cells, the whole group retaining for a time the size and shape of the mother cell (fig. 7). If cut transversely, this group is seen to consist of about 10–20 cells (fig. 8). The young strand at this stage is thus a series of such cell groups, each derived from a single mother cell.

As the fruit expands, these elongate cells grow in length though but little in width. The surrounding parenchyma cells continue to divide. Thus series of long narrow cells, connecting with others to form the young strands, may now be seen in the midst of isodiametric parenchyma cells. For considerable time the groups of cells which were formed from a single mother cell may be distinguished, since they are of the same length (figs. 9, 10). Their end walls, however, instead of being transverse are now becoming pointed, and many of the cells begin to show some resemblance to fibers. During this period longitudinal divisions may occur, so that the number of cells in a strand increases somewhat (fig. 11). Some transverse divisions are also found. The parenchyma cells immediately adjacent to the fiber strand divide more frequently than the others, so that a sheath of relatively small cells is differentiated around the strand. In some cases there is evidence that these cells may actually add to the size of the strand.

Relatively early in development some internal differentiation also arises in the strand itself. Several of the innermost cells begin to take on the character of

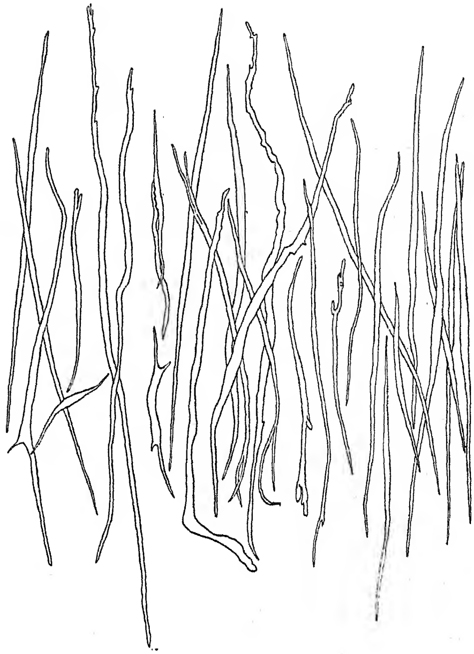


Fig. 14.—Typical fibers isolated by maceration from outer transverse strand in Line A5. Ovary diameter 100 mm. Both straight and forked or branched forms are shown, some of which are short elements from strand periphery, forming transitions to sclerified parenchyma cells. Longest fibers are about 3600 μ long and 55 μ wide. $\times 24$.

phloem elements. In the larger bundles one or more ringed or spiral vessels make their appearance.

In a typical fibrous strand almost all the cells ultimately become thick-walled, elongate fibers (figs. 12, 14). Their growth in length at first keeps step exactly with the increase in size of the fruit itself. This may be shown by measuring the length of typical young fiber initials

from comparable strands and plotting them against the width (or length) of the ovary in which they occur. After these initials reach a length of about 200 μ, it becomes evident that they are growing in length faster than the dimensions of the fruit. Measurements of the fibers in the maturing fruit are not easy to obtain, since an entire cell can rarely be observed in a single section, and since they are still too delicate to macerate. As they cease growing, however, these fibers develop thick and heavily lignified walls and can be separated by maceration. Measurements at this time show them to have reached a much greater length than could be accounted for by expansion at the same rate as the fruit. Thus in a certain race fiber initials from the outer wall were on the average 30 μ long in ovaries 2.5 mm. wide, 60 μ in those 6 mm. wide (about the time of flowering), and 250 μ in those 25 mm. wide. Fibers from these same strands at maturity were 2500 μ long in fruits 100 mm. wide, whereas they would have been only about 1000 μ long if they had continued to increase at their earlier rate, corresponding to that of the fruit. This must mean that the developing fibers continue to elongate after the fruit has slowed down or stopped and that their ends grow past each other intrusively (6). This conclusion is also supported by the fact that the number of fibers seen in cross section of a mature strand is considerably greater (twice or more) than the number in a young strand (fig. 12 as compared with fig. 11).

Thus intrusion evidently takes place before the heavy lignified wall is formed. That it occurs chiefly at the ends of the fibers is suggested by the fact that at maturity the ends are very much thinner than the middle regions. The ends are also frequently forked, as though elongation had been blocked. The whole fiber is often gnarled and irregular in shape, with projecting portions at various points, as though the whole wall at one time had been more or less plastic and had grown into any cavity adjacent to it. These irregular shapes occur most frequently in those parts of a strand which branch or are sharply bent. Here also the fiber ends are often twisted around each other (fig. 14). Much of the increase transversely in cell number of the strands is probably due to the presence of these branched ends of the fibers. The very acute angles of the fiber walls, as seen in cross section, are quite different from those of normal tissue and further suggest that many of these cells have reached their present position by intrusion (fig. 13).

Strands in the same region may differ considerably in the length of their fibers. Those with shorter fibers are presumably the ones which differentiated later from ground parenchyma, and in many cases this can be shown to be true. Thus the small strands in the outer wall and those arising as secondary anastomoses in the inner region, both of which appear relatively late, have the shortest fibers of all.

During their development the diameter of the fiber elements increases somewhat. At their first differentiation they are approximately 3 μ wide and at maturity 25–50 μ wide. The stouter strands tend to have somewhat wider cells, though the chief factor in strand diameter is the number of its cells.

The thickness of the fiber walls does not vary considerably in various parts of the fibrous net. Conspicuous thickening of the wall does not occur until the fibers cease growing. The walls are often markedly thinner near the ends of the fibers, indicating that thickening began later here than in the middle. Lignification does not proceed at the same time every-

where but occurs in some strands before others and in some portions of a strand earlier than in others. In all parts of the net the fibers were so tightly cemented together that only a drastic maceration technique was able to separate them.

The general character of the net and its constituent fibers seems to be essentially the same in parthenocarpic fruits as in those with seeds, although in the former the fruit and its net are usually somewhat smaller.

In the very elongate fruits pollination was often not complete, with the result that only the apical portion of the fruit produced seeds and grew to its normal diameter. The basal portion was relatively slender, but its fibrous skeleton, although relatively small, was found to resemble that of the fertilized portion. Its strands were markedly narrower and its fibrous cells smaller, however. Development was evidently arrested and maturity was reached before normal size had been attained.

The various races studied differ somewhat in the character of their fibers. In most of them, such as A5 and B10, the fibers reach a length of 3500–3800 μ (average 2400 μ), but in a few cases—such as B2—no fibers longer than 1900 μ were found, and many were shorter. This is a small-fruited line with a rather delicate net. Its fibers could also be macerated with greater ease than in the other lines. In general there tends to be a relation between small fruit size, delicate fibrous net, and shorter fibers. Degree of lignification does not seem to be related to fruit size.

Discussion

The development of the fibrous net of *Luffa* is a good example of the way in which a bundle system may become differentiated in the midst of relatively undifferentiated parenchymatous tissue. This may often be observed in other plants in the origin of the veins and fiber strands in leaves and fruits. Sometimes differentiation begins very early, going back almost to the start of growth. In other cases, as in that of the fibrous net here described, it may not begin until the general form of the organ and the demarcation of its various regions have been attained. The differentiation of the net bears little visible relation to this earlier differentiation, the strands twisting and branching among the orderly rows of the parenchyma cells in the young ovary primordium without relation to the direction of these rows or the shape of their cells. It is almost as though one pattern had been superposed on another.

In such cases, relatively large and often highly vacuolate parenchyma cells, if they happen to lie in the course of one of these developing strands, will become meristematic again and divide actively although their neighboring cells go on to maturity undivided. These illustrate the fact recently emphasized (7) that division in embryonic tissue is by no means limited to small, richly protoplasmic cells but often takes place in large and vacuolate ones. This differentiation also recalls the development of strands of xylem and phloem in parenchymatous tissue through regeneration, as described by Freundlich (1) and others, and the origin of vascular connections between adventitious buds and deeply seated bundles, as shown by various writers.

Other members of the Cucurbitaceae show similar development of a vascular net, but in most of the genera—such as the various squashes, melons, and cucumbers—the strands consist only of phloem and parenchyma, the latter of relatively long but unlignified cells. These strands are most conspicuous in

the coarse and "stringy" pumpkins but are present even in fruits with the most delicate flesh.

Continued elongation of fibers after their adjacent cells have stopped growing is not very common. TAMMES (8), MEEUSE (4), and MAJUMDAR (3), working with fibers and collenchyma of various sorts, have found that the elongation of most of these cells does no more than keep pace with the elongation of the organ of which they are a part. On the other hand, many cases are known where sclereids of various sorts elongate enormously, growing in between the cells of adjacent tissues for relatively great distances. VAN TIEGHEM (9) has described such cells in the leaves of Peneaceae and JADIN (2) in those of Simarubaceae, and numerous other cases are known. BLOCH has recently studied their development in the air roots of *Monstera*. Some of the greater length of such pointed cells may be accounted for by differential wall growth, by means of which their ends become progressively more acute. In such extreme cases as the present one, however, it must be due to the continuance of growth by one cell after its neighbors have stopped growing or are elongating in an opposite direction. Such growth seems to be of the type called intrusive by SINNOTT and BLOCH (6), where one region of a cell, usually its tip, actually grows in between others, much as does a tylosis, rather than by gliding over the surface of adjacent cells. That growth of the *Luffa* fibers is chiefly at their ends is indicated by the much thinner walls there. The two halves of a pit are always opposite each other, showing that there is no gliding after the pits are formed. Critical evidence from other sources that cells do not glide makes it unlikely that these fibers and sclereids are exceptional in their behavior.

The intrusive growth of *Luffa* fibers appears to be associated with their specialized character, since in the corresponding strands of other cucurbits, where the cells remain thin walled these never seem to attain greater length than could be accounted for by growth of the entire organ.

The morphological nature of the *Luffa* strands is also a matter of some interest. There are transitions of various sorts from the typical fibrovascular bundles of the outer wall to the typical strands of the fiber net. The latter seem to be vascular strands in which the xylem and phloem have been much reduced (the xylem often wholly absent) and in which the fibrous tissue is greatly developed. Their conductive function, which presumably was primitive, has here largely been replaced by a mechanical one involved in the support and dispersal of the seeds.

No sharp line can be drawn between the typical vascular cells in these strands and those which belong to the surrounding ground tissue. Cells transitional in shape and pitting between xylem cells and fibers are not uncommon. Even more frequent are cells intermediate between typical fibers, which presumably are "vascular" in origin, and the relatively short sclerenchymatous cells of the ground tissue. Such transitions are common in the outer wall, where most of the ground tissue is sclerified. They may also be seen at the surface of many of the strands, where the cells of the sheath, intermediate in shape between parenchyma and fibers, often have thick lignified walls. Such cases demonstrate the impossibility of drawing sharp lines between categories of tissues, such as "vascular" and "fundamental." The character of each cell seems to be determined by the position it occupies in a developmen-

tal pattern rather than by its membership in a particular lineage or category of cells.

The usefulness of the *Luffa* fiber depends in part on the fact that its strands form a continuous and closed network. The diameter of the strands and their number per unit volume make this network relatively coarse or fine. The durability of its fibers depends upon the degree of lignification of their walls and on the strength with which the cells are bound together by the intercellular substance. All these traits, as well as the size and shape of the net, show considerable genetic variability and may be made the basis for experiments in breeding and selection. They could doubtless be modified considerably by this means, and types of sponges could be developed which would have many more economic uses than *Luffa* does at present.

Summary

1. The development of the fibrous network is described for a number of races of *Luffa cylindrica*, the "vegetable sponge."

2. The strands begin to differentiate when the ovary primordium is about 1 mm. wide, and new ones continue to form until cell division ceases. A complex anastomosing network is produced.

3. The first indication of the appearance of a new strand is the increase in protoplasmic content of a row of parenchyma cells. These then divide parallel to the axis of the future strand but with no necessary relation to the shape of the mother cells or to the pattern of the ground tissue.

4. The cells thus formed develop into the fibers. They elongate as the surrounding parenchyma grows but continue to grow intrusively, chiefly at their tips, for some time after fruit growth ceases.

5. Most strands consist chiefly of fibers, but there are a few phloem elements in the middle of each and often one or more xylem cells. The strand is a modified vascular bundle.

6. Fibers range in length from very short ones to about 3800 μ, being on the average 1500–2000 μ long. Many are branched or have irregular shapes. They are very difficult to separate by maceration.

7. The various races differ considerably in the development of the fibrous net, in the diameter and spacing of its strands, and in the length and character of the fibers.

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LITERATURE CITED

1. Freundlich, H. F., Entwicklung und Regeneration von Gefässbündeln in Blattgebilden. Jahrb. wiss. Bot. 46:137–206. 1909.
2. Jadin, F., Contribution à l'étude des Simarubacées. Ann. Sci. Nat. 8 Sér. Bot. 13:201–304. 1901.
3. Majumdar, G. P., The collenchyma of *Heracleum sphondylium* L. Proc. Phil. Soc. Leeds, Sci. Sec. 4:25–41. 1941.
4. Meeuse, A. D. J., Development and growth of the sclerenchyma fibers and some remarks on the development of the tracheids in some monocotyledons. Rec. Trav. Bot. Néerl. 35:288–321. 1938.
5. Sinnott, E. W., A developmental analysis of the relation between cell size and fruit size in cucurbits. Amer. Jour. Bot. 26:179–189. 1939.
6. Sinnott, E. W., and Bloch, R., Changes in intercellular relationships during the growth and differentiation of living plant tissues. Amer. Jour. Bot. 26:625–634. 1939.
7. ———, and ———, Division in vacuolate plant cells. Amer. Jour. Bot. 28:225–232. 1941.
8. Tammes, T., Der Flachsstengel. Haarlem. 1907.
9. Van Tieghem, Ph., Recherches sur le structure et les affinités des Thyméléacées et des Pénéacées. Ann. Sci. Nat. 7 Sér. Bot. 17:187–294. 1893.

EFFECTS OF PHOTOPERIOD AND TEMPERATURE ON GROWTH AND DEVELOPMENT OF KOK-SAGHYZ

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Introduction

The influence of photoperiod on the growth and development of *Taraxacum kok-saghyz* Rodin has been determined to provide information that may be of use in establishing the production of this crop on an economical basis in this country. The length of the photoperiods and the temperature at which they are applied are important factors in the production of many crops, since they limit the range in which the plants can be grown, influence the time of flowering, control the vegetative development of the plant, and affect its chemical composition.

Since little was then known concerning the photoperiodic and other environmental requirements of kok-saghyz, the first shipment of seed from U.S.S.R. was distributed by the Rubber Plant Investigations Project for testing in certain localities of the United States and Canada where photoperiodic and other environmental conditions were similar to those prevailing in that part of central Asia where the plant is native(1). In addition to these studies under natural environment, investigations of the effects of photoperiod and of the interaction of photoperiod and temperature on the growth and development of this plant were conducted at Beltsville by the Photoperiodic Project.[4] Knowledge of the nature and extent of the response of kok-saghyz to these factors should be useful in the selection of the most promising localities in which to establish this crop and may suggest methods of culture which will speed the development of new types and may aid in the selection of those progenies best adapted to a particular region.

Experimental results

Plants for these studies were secured from field plantings in various states and from seedlings grown in the greenhouse. All the seedlings were started in flats and transplanted when they had two or three leaves. They grew well under the various experimental conditions and comparatively few were lost.

Greenhouse experiments.—Photoperiods of 8, 10, 12, 14, 16, and 18 hours were maintained. The natural photoperiod was extended with Mazda light of about 50 foot-candles, and the individual lots of plants were covered with a double layer of black sateen at the close of their respective photoperiods. These covers were removed at 8:00 A.M. the following morning.

The first greenhouse experiment was concerned with the influence of photoperiod on the development of flower buds that had been previously initiated on plants growing in the field.

A supply of plants was obtained during January, 1943, from St. Paul, Minnesota; Parma, Idaho; Klamath Falls, Oregon; Berkeley, California; and from Beltsville, Maryland. Represent-

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ative plants from each source were examined with a wide-field binocular microscope, and flower primordia were found to be present on almost every plant. These primordia were in the very earliest recognizable stages of development on some plants but had developed to a size easily visible with the naked eye on others.

Plants from each locality were potted in 6-inch pots and subjected to the six photoperiods being maintained. Ten to twenty plants from each locality were placed on each photoperiod. The plants quickly resumed growth and within a few weeks all of them flowered. The lots on 8-and 10-hour photoperiods were slightly later in blooming than the others and formed somewhat shorter flower stalks, but these differences were not sufficiently great to be considered significant.

In this experiment relatively little seed was formed on any of the plants that flowered unless the flowers were artificially cross-pollinated. Since seed was formed abundantly on all lots following hand cross-pollination, it seems evident that the photoperiods were in no way responsible for the lack of seed production. WARMKE (2) has found that there is a high degree of self-sterility in kok-saghyz and that in screened greenhouses, or when insects are scarce, lack of cross-pollination is the main cause of low seed setting.

The results of this experiment indicate that growth and development of flower buds initiated in the field before the treatments were started were not inhibited by any of the photoperiods employed. The range in length of these photoperiods was about as great as would be encountered in the field in any part of the United States during the growing season. So far as photoperiod alone is concerned, it would therefore appear feasible to move plants from one section of the country to another for seed-production purposes if it were desirable to do so.

Further greenhouse experiments were concerned with the influence of photoperiod on the initiation of flower primordia in seedlings. The seedlings were started in January, 1943, from seed obtained from the Rubber Investigations Project of the Bureau of Plant Industry, Soils, and Agricultural Engineering. The seed was secured from Russia through the offices of the State Department.

From the time of planting the seedlings were maintained on daily photoperiods of 8, 10, 12, 14, 16, and 18 hours. As soon as the seedlings had developed two or three small leaves they were transplanted to 2-inch pots, in which they were grown until they had produced rosettes slightly larger than the diameter of the pot. The plants were then shifted to 4-inch pots. The experiment was replicated three times. Replicates 1 and 2 were in a single compartment of the greenhouse and replicate 3 was in an adjoining compartment. During most of the experiment the temperature of the latter compartment was maintained about 5° F. warmer than that of the former.

All the plants made satisfactory growth, but those receiving photoperiods of 12 hours or more developed slightly larger rosettes and appeared somewhat more vigorous than those on the two short photoperiods. The leaves of the plants receiving long photoperiods grew semi-erect, in contrast to the prostrate leaves of the plants on short photoperiods.

Dissections were made of two plants from each photoperiod on February 27, when the plants were about 6 weeks old, to determine the earliest time at

which flower primordia were initiated. The stem terminals of all these plants were forming vegetative leaves, and no flower primordia were present. A week later microscopic flower buds were present on two of twelve plants dissected. Dissections were discontinued at this time because data concerning subsequent development of the plants were desired. Further data on floral initiation were based on the time of appearance of macroscopic buds.

These buds were visible in various stages of development on many plants on March 20. Flower buds appeared on 174 plants, or 33 per cent of the plants of the experiment, during the period March 20 to May 8, and about two-thirds of these were present before April 1. Their formation had nearly ceased by May 8, only five plants being recorded during the week immediately preceding this date. The percentages of plants forming flower buds on photoperiods of 8, 10, 12, 14, 16, and 18 hours were 15, 24, 36, 37, 48, and 37, respectively. The percentages for replicates 1, 2, and 3 were 42, 38, and 20. Variance analysis of the data indicates that the differences between the photoperiods and also between the replicates were significant at the 5 per cent level but not at the 1 per cent point.

Flowering was not completely inhibited by any of the photoperiods, although the data suggest that the 8- and 10-hour photoperiods were somewhat less favorable than the four longer ones.

The low percentage obtained in replicate 3 may have resulted from the somewhat higher temperature of that section of the greenhouse. Further data relating to the influence of temperature will be presented later.

During February a second lot of seed was planted for a repetition of the preceding experiment. In this second greenhouse experiment the same photoperiods were tested as in the previous one. The experiment was set up in duplicate instead of triplicate, with the two replications in the same greenhouse compartment. Two soil types, one a composted topsoil from Beltsville and the other a highly alkaline one from Oregon, on which a very satisfactory crop of kok-saghyz had been grown the previous season, were employed. The pH of the Beltsville soil was 5.8 and that of the Oregon soil 9.05. The number of plants per sub-lot was 50, or a total of 1200 in the entire experiment.

All the seedlings grew very well, but those in the Beltsville soil were considerably more vigorous than those in the western soil. As in the previous experiment, larger, more erect growth was again observed in the plants subjected to longer photoperiods. Only 43 of the 1200 plants of the experiment formed visible flower buds before June 1, and all but four of these were plants growing in Beltsville soil. Only one plant of those receiving 8-hour photoperiods and only six of those on 10-hour photoperiods formed flower buds. The remaining 36 plants were about equally divided among the four longer photoperiods.

This experiment was started about a month later than the preceding one, and the higher temperatures then prevailing may have been responsible for the very much lower percentage of plants forming flower buds.

Results of the two experiments indicate that floral initiation occurs in seedlings subjected to any of the photoperiods tested but is somewhat more abundant on plants receiving photoperiods of 12 hours or more.

GROWTH-CHAMBER EXPERIMENTS.—Two experiments were conducted in

growth chambers concurrently with the experiments in the greenhouse. In these a study was made of the interaction of temperature and photoperiod on growth and development of the plant.

For the first experiment the temperatures of the four available chambers were adjusted to 55°, 65°, 75°, and 85° F., respectively. The chambers were illuminated with a combination of carbon arc and Mazda light. The intensity of the Mazda light in all chambers at the plant surface was about 160 foot-candles; that of the arc light in chambers 1 and 2 was about 1200; and that in chambers 3 and 4 about 1800.

Photoperiods of 10, 13, and 16 hours were obtained in each chamber by covering certain plants with lightproof covers at appropriate times. The operation of the Mazda and arc lights was controlled by means of electric time-switches set to turn the lights on at 8:00 A.M. and off at midnight. The 10-hour plants were covered at 6:00 P.M. and the 13-hour ones at 9:00 P.M.

Seedlings were started in the greenhouse on photoperiods of 10, 13, and 16 hours, respectively. As soon as they had two or three leaves they were transplanted to 3-inch pots filled with composted soil and transferred to the growth chambers. The plants were held in these chambers from February 5 to March 8, 1943. The experiment was discontinued on March 8, following a failure of mechanical equipment in the 65° room which resulted in loss of the 10- and 13-hour plants and in slight damage to the 16-hour ones. All the plants at this time were moved into a greenhouse, where they received photoperiods of 16 hours or more daily until May 6.

Pairs of plants from each temperature and photoperiod were photographed on March 4, after 29 days in the growth chambers (fig. 1). Considerable variability existed within each lot, and the plants photographed were selected to represent as nearly as possible the average size for the lot.

The largest plants in each chamber were associated with the longest photoperiod. This difference between plants of different photoperiods was most prominent in the 55° and 65° chambers and least in the 75°. The intensity of radiation in each room was of course constant throughout the period of illumination, so that the total amount of radiant energy received by plants of different photoperiods in any room was in the same relationship as the daily duration of light received by them. Since the size of the plants on the different photoperiods is thus correlated with duration of illumination and with total amount of radiation, the differences between them may have resulted primarily from either of these factors.

The plants produced in the 75° and 85° chambers were characterized by a prostrate habit and gray-green color, while those of the two lower temperatures were of a yellow-green color and their leaves were semi-erect. These differences in color and growth habit may have resulted from differences either in temperature or in light intensity, because the rooms at the two higher temperatures were equipped with arc lights of considerably higher intensity than those of the other two rooms. The differences, whatever their cause, persisted long after the treatments at different temperatures and photoperiods were discontinued and were still evident when the plants were finally discarded on May 6.

Although the plants did not flower abundantly, differences associated with both temperature and photoperiod were

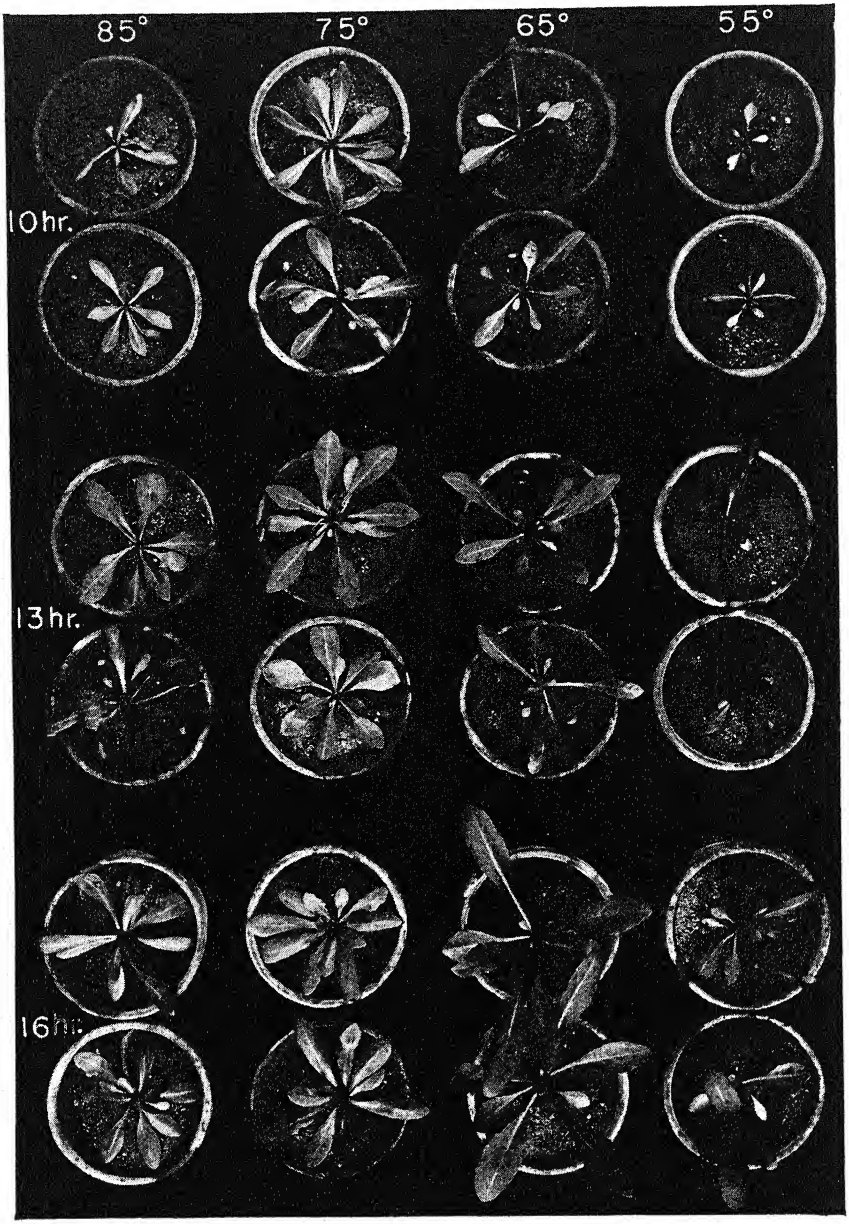

FIG. 1.—Kok-saghyz seedlings after 29 days in growth chambers at temperatures indicated and at daily photoperiods shown. Only two or three small leaves present on seedlings at beginning of experiment.

apparent. In the 55°, 75°, and 85° lots, flowers were formed by six, eight, and twenty-nine plants, respectively, and at photoperiods of 10, 13, and 16 hours on seven, six, and thirty plants, respectively. The greatest number of flowers in any one treatment was in the 55° lot that received 16-hour photoperiod. Although the 65° plants held on 16-hour photoperiod were continued in the greenhouse, no data are presented from them because they had been damaged before their removal to the greenhouse.

The results of this experiment suggest that low temperature and long photoperiod are favorable to the initiation of flower buds by kok-saghyz seedlings.

The second experiment in the growth chambers was started on March 10 and continued until April 27. In this experiment 12- and 16-hour photoperiods were maintained at temperatures of 65° and 75°F., and the plants were grown in two different types of soil under each of these conditions. The soil types were a composted topsoil from Beltsville and an alkaline soil from Oregon. This experiment was performed in duplicate since two chambers were available for each temperature. The temperatures of the various chambers were so arranged that each replicate included one with a high-intensity arc light and one with a low one. This was done to determine whether or not the prostrate habit of the plants grown in rooms 3 and 4 of the previous experiment resulted mainly from the higher temperature or from other characteristics of those chambers.

In each chamber the plants were divided into four main groups of eighty plants, forty in Beltsville soil and forty in Oregon soil. Two of the groups received 12-hour photoperiods and the other two 16-hour.

Growth of plants under all the experimental conditions was very good, but the plants in the Beltsville soil were somewhat larger than those in the Oregon soil. The prostrate habit was again prominent in chambers 3 and 4. Since one of these chambers was operated at 65° in this experiment it appears probable that the prostrate habit of growth observed in the former experiment was in some way correlated with conditions in the chambers other than temperature.

While the plants were still in the growth chambers, forty-two of them formed flower buds and a few blossomed. These forty-two plants were distributed among the various treatments as follows: thirty-three were in Beltsville soil and nine in Oregon soil; thirty-five received 16-hour photoperiod and seven received 12-hour; sixteen were at 65° and twenty-six at 75°F.

In this experiment the plants remained in the growth chambers 18 days longer than in the previous one. Formation of flower buds was not observed in the first experiment while the plants were in the chambers, but in the second experiment flower buds were visible on many of the plants before they were removed. In the first experiment flowering was greater in the lots that had received the lower temperatures, while in the second the reverse effect was observed. The data in the former were obtained from plants a few weeks after the treatments with photoperiod and temperature had been discontinued and in the latter while the treatments were still in progress. The apparent reversal disappeared, however, after the plants were removed to the field. Further data on this point are presented in the next section.

OUTDOOR PLANTINGS.—Plants fr certain of the experiments in the gr

house and growth chambers were transplanted outside, so that any effects of treatment that appeared later in their development might be observed. Three such plantings were made, one from the second growth-chamber experiment and two from greenhouse experiments.

The plants of the second growth-chamber experiment that had not produced visible buds on April 27, the date on which the treatments with temperature and photoperiod were discontinued, were transplanted to the field.

By June 4 a total of seventy-nine plants had developed visible buds or had flowered in the field. Sixty-seven of these were from the chambers that had been operated at 65° and twelve were from the 75° chambers. By July 3, fifty-three additional plants from the low temperature chambers and thirty-three from the higher temperature chambers had formed buds, making a total of 119 and forty-five, respectively. Apparently some effect of the lower of the two temperature treatments applied in the growth chambers was expressed in the later development of the plants and resulted in the formation of flower buds on a greater number of them. No such effect resulting from photoperiodic treatment was observed.

The plants for the second outdoor experiment were grown in the greenhouse from March 10 to April 17 under the six conditions of photoperiod of the greenhouse experiments. During this time a few of the seedlings bloomed, but toward the end of the period blooming had nearly ceased and most of the plants had discontinued expanding new leaves. Since this condition did not occur in the growth-chamber experiments, it was thought that possibly the high temperatures in the greenhouse during the daytime inhibited their development. Consequently on April 17 most of these plants were transferred to the growth chambers, where part of them were held at 65° and the rest at 75° until May 4. A few plants from each photoperiod were kept in the greenhouse as controls. On May 4 all the lots, totaling 960 plants, were transplanted to the field. During the next 2 months only nineteen plants of this entire planting formed flower buds. Most of the plants grew very little during this 2-month period. Occasional plants developed vigorous rosettes but failed to form flower buds, even though the plants were considerably larger than others that blossomed. The few plants that flowered were not restricted to any particular treatment.

Another lot of plants that had received approximately the same treatment in the greenhouse as those of the second outdoor experiment constituted the material for the third outdoor experiment.

These seedling plants were started in the greenhouse in 2-inch pots in early February on six different photoperiods replicated three times. They were grown in these pots for approximately 2 months during which time a few plants in some of the lots formed buds or bloomed. On April 6 all the lots were moved into another greenhouse, where they received the natural photoperiods then prevailing. On April 10, twenty plants from every lot, or a total of sixty from each of the six photoperiods, were transplanted outdoors into boxes of soil $1 \times 1 \times 3\frac{1}{2}$ feet in size and filled with well-composted soil. Ten plants were transplanted to each box. On April 20 a similar lot of plants was also transplanted.

The 10-day interval between the two plantings was much colder than the 10-day period following the second planting. The maximum temperature during

this first period was 64°, and on two days it was 42° and 43°, respectively. Minimum temperatures were below freezing on three nights and were less than 10° above freezing on five other nights. During the 10-day period from April 20 to 29, the maximum temperature was 79° and the lowest maximum any day was 55°. No freezing nights occurred, and on only six nights was the temperature less than 10° above freezing. Minimum temperatures after April 29 were nearly all above 50° F. Both lots thus received low temperatures after they were transplanted, but the first lot received considerably more cold than the second.

On May 27, 47 days after the first transplanting, flower buds or flowers were visible on 88 per cent of the first lot and on 59 per cent of the second. On June 25 these percentages had risen to 98 and 88, respectively. These results suggest that possibly the low temperatures received shortly after transplanting may have stimulated the formation of flowers. No differences in time or percentage of bloom were correlated with any of the treatments received prior to transplanting.

Summary

1. Flower primordia were present on plants of *Taraxacum kok-saghyz* collected from several localities in the central or northern parts of the United States during the late fall or winter months. Photoperiods of 8, 10, 12, 14, 16, and 18 hours did not inhibit the development of these primordia into mature flowers when the plants were transplanted in the greenhouses.

2. Wide variability of leaf pattern was observed in the seedlings, but morphological characteristics of the flowers indicated that almost all were kok-saghyz plants. Seedling plants subjected to six different lengths of photoperiods varying from 8 to 18 hours flowered under all conditions, but more of them flowered with photoperiods of 12 hours or longer than with shorter photoperiods.

3. Plants that received low temperatures during their early development flowered more abundantly than those that received no low temperature. Ninety-eight per cent of one lot of seedlings that had received several weeks of cold weather during the spring flowered, while less than 2 per cent flowered in a similar lot that received no low temperature.

4. Results of these experiments indicate cool temperature and long photoperiod as conditions most favorable to early blooming of seedlings.

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LITERATURE CITED

1. Brandes, E. W., Rubber from the Russian dandelion. Agriculture in the Americas 2: 127–130. 1942.
2. Warmke, H. E., Macrosporogenesis, fertilization, and early embryology of *Taraxacum kok-saghyz*. Bull. Torr. Bot. Club 70: 164–173. 1943.

SECONDARY ASSOCIATION OF FRAGMENT CHROMOSOMES IN GENERATIVE NUCLEUS OF TRADESCANTIA AND ITS BEARING ON THEIR ORIGIN[1]

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Introduction

Many plant and animal species are known to possess fragment chromosomes in addition to their normal complement (6, table 16). In *Tradescantia* they are rather widespread, particularly in the *virginiana* group, and more especially in those species closely related to *T. paludosa* (1, 4, 5, 9, 14, 17). These fragments, in *Tradescantia*, measure about one-sixth to one-ninth the length of the major chromosomes, possess a centromere, and pass through the division cycle with little irregularity. So far as is known, they exert no genetic influence, for as ANDERSON and SAX (1) point out, as many as twelve can be present in a single plant with no appreciable effect. In this respect they behave much as the B-chromosomes in maize, and like them they are undoubtedly composed to a large extent of heterochromatin.

There is some disagreement as to their structure. WHITAKER (17) has considered them to possess a terminal centromere. In some species inversion crossing-over has revealed the fragments to be atelomitic, with the centromere nearly median (9, 14). Since in interspecific hybrids (for example, *T. paludosa* × *virginiana* or *T. paludosa* × *canaliculata*) the fragments pair readily (SWANSON, unpublished), this seems to be satisfactory ground for assuming that throughout the genus they are all structurally similar, recognizing, however, that the fragment can change shape and size through crossing-over (5). Recent findings on the instability of the truly terminal centromere (7, 12) also tend to support the contention that the centromere is not terminal, and additional support for this is gained from this study of the fragment in the pollen tube, where the morphology is almost diagrammatic.

The origin of the fragment chromosomes in *Tradescantia* has not been satisfactorily determined. That they are homologous, at least in part, with the major chromosomes there seems no doubt (5, 9, 14, 17), since pairing at meiosis has been frequently observed between them and the major chromosomes. WHITAKER (17), however, finds no such pairing in *T. paludosa*, the species studied here. Meiotic association has been most frequently detected at terminal regions of the major chromosomes, although lateral pairing in the median region of the arms (4) and centric pairing (14) have also been reported. It is not known whether such associations are the result of true chiasmata, such as DARLINGTON (5) has shown can be produced terminally. As to their origin, two tentative suggestions have been advanced. Basing his hypothesis on the fact that no terminal pairing was found at meiosis, WHITAKER (17) concludes that they have resulted from a fragmentation process which eliminated a major portion of both arms. On the other hand, SWANSON (14), finding both distal and proximal pairing at meiosis, suggests that they might have arisen from crossing-over in overlapping inversions, thus preserving both the centric and terminal regions intact while eliminating the median portions of both arms.

Secondary association provides a further means of attacking this problem. This type of loose pairing is most generally found at meiosis in organisms with small chromosomes (3, 10, 16), and wherever existing, it presents a means of rec-

[1] Journal article no. 627, new series, Agricultural Experiment Station, East Lansing, Michigan.

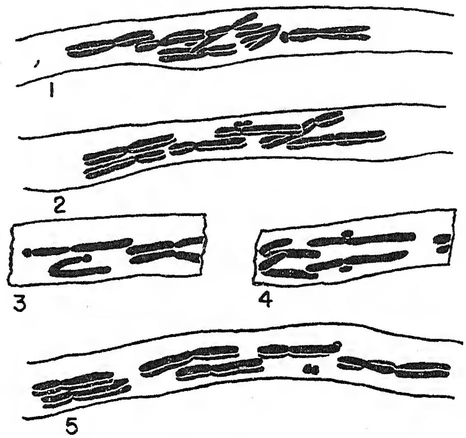
ognizing homologies. It is found less frequently in somatic cells, but a recent study of the pollen-tube chromosomes in *Tradescantia* has shown that the fragments reveal a marked tendency to associate with the major chromosomes at centric and terminal regions. This paper deals with this particular aspect of the problem and its bearing on the origin of the fragments.

A diploid (2n = 12) clone of *T. paludosa* Anders. & Woodson, possessing two fragment chromosomes, furnished the material for this study. The pollen tubes were grown on an agar-gelatine-sugar medium (15), with enough colchicine replacing the acenaphthene to give a 0.001 per cent solution. The colchicine seemed in no way to affect the secondary association but merely served to spread the major chromosomes in the pollen tube, thus facilitating the analysis. All preparations were fixed for 10 seconds in alcohol-acetic and then stained with acetocarmine.

Observations

Since the compactness of prophase in the pollen tubes is such that it prohibits a detailed analysis, only metaphase configurations were studied to determine the pairing relationships with the major chromosomes. Each fragment was classified in one of three categories (table 1). When paired terminally, the fragment was closely attached to the end of the chromosome arm (fig. 1); when paired at the centromere, it was usually so arranged that the axes of both fragment and major chromosome were parallel (fig. 2). All other fragments were considered to be distributed at random, although it was evident that many of them were loosely associated with a particular chromosome. Those definitely classified therefore represent the minimum number showing secondary association.

A total of 800 fragments were studied, these being taken from several permanent preparations made at various times. The percentages of paired associations varied widely among the slides, being as high as 50 per cent in some instances and



Figs. 1–5.—Fig. 1, fragment paired terminally; fig. 2, fragment paired at centromere; figs. 3, 4, separation of fragment and major chromosome with persistent secondary association; fig. 5, normal complement of chromosomes; fragment "at random."

as low as 28 per cent in others, with an average of 42 per cent (table 1). This distribution is obviously not at random, particularly since it became clear that

TABLE 1

Distribution of fragments in pollen tubes of T. paludosa

At random	Terminal pairing	Centric pairing	Total
463	169	168	800

the fragments were associated with a particular kind of major chromosome.

The haploid complement in *T. paludosa* consists of two isobrachial and four heterobrachial chromosomes (fig. 3). Within these two groups it has not been possible to distinguish individual chromosomes, but terminal pairing of the fragment always involved the shorter arm of one of the heterobrachial chromosomes, while the same one or one similar to it was invariably associated with the fragment whenever centric pairing oc-

curred. While it is impossible to state that the pairing in each instance involved the same chromosome, a study of those nuclei possessing two fragments makes this a reasonable assumption. In no case was a nucleus with two fragments found in which both were paired terminally, although one nucleus was observed in which two fragments were paired at the centromeres of different chromosomes, both heterobrachial. This could be taken to mean that centric pairing was less specific than terminal pairing, or that two of the major chromosomes shared a region in common homologous with a portion of the fragment. No nucleus was found with one fragment paired terminally and the other at the centric regions, thus making it uncertain whether the major chromosome involved in terminal pairing is the same as that showing centric pairing.

The terminal and centric associations frequently persisted, even after the chromatids had become widely separated (figs. 4, 5); but there is no evidence for believing that the union was other than temporary or that it involved somatic crossing-over.

Discussion

DARLINGTON (3) first realized the importance of secondary association as a means of identifying homologies, and he has used this method in determining the degree and kind of polyploidy in such genera as *Prunus*. LAWRENCE (10) working with *Dahlia*, and UPCOTT (16) with *Aesculus*, have reported similar successes. In somatic cells, exclusive of dipteran salivary glands, this phenomenon has best been shown in the Diptera (11), and STURTEVANT and DOBSHANSKY (13) found, through the use of translocations, that it is dependent upon a part-for-part homology. Besides the secondary association shown in this study, the only other instance known to the writer is that in the somatic cells of *Dahlia* (10), although here the association is radial rather than parallel. A study of microspores and root-tip cells in *Tradescantia* revealed no pairing of any kind, and it seems plausible to assume that the limitations of space in the pollen tube permit the forces of attraction to function more effectively.

The pairing evidence supports the conclusions previously drawn from meiotic data (14) that the fragment is homologous with one or more of the major chromosomes and that the homologous portions are at the distal and proximal regions of the chromosome arms. More specifically, it can now be shown that one end of the fragment is homologous with a terminal region of one of the heterobrachial chromosomes, and also that another portion of the fragment, probably in the neighborhood of the centromere, is homologous with centric regions in one and possibly two of the heterobrachial chromosomes. It appears reasonable to assume that these pairings are specific as to homology. Of course there remains the possibility that these regions are heterochromatic, in both the fragment and major chromosomes, and that their pairing is of a nonspecific kind; but in view of the specificity of somatic pairing (13) and the lack of evidence pointing to a somatic pairing of heterochromatic regions, this explanation appears unlikely.

These conclusions regarding the structural affinities of the fragment are not in agreement with the conclusions of ANDERSON and SAX (1) and WHITAKER (17), who feel that the fragment represents a median segment of one of the larger chromosomes. On the other hand, it is possible that the fragments dealt with are structurally different, for as DARLINGTON (5) points out, a fragment may, through crossing-over, undergo a change in shape

and size. However, the fact that hybridization studies show the fragments in different species to be very similar, and the fact that WHITAKER studied the same species used here, indicate that the fragments are in all probability structurally alike.

Granting that the fragment is derived from a major chromosome, the diminution of chromatin can result from several processes: fragmentation, intercalary deletion, overlapping inversions, and translocation. The first can be eliminated without further consideration, since the evidence that the terminal regions of the fragment and one of the major chromosomes are homologous indicates that simple fragmentation, or loss of the distal portion of the chromosome arm, could not lead to the structure observed.

Each of the other three methods, or a combination of them, could lead to the origination of a fragment such as that found in *Tradescantia*, that is, one where the median regions of at least one arm have been eliminated while the terminal and centric portions remain intact. The data, of course, permit a discussion of the derivation of only one end and some other portion of the fragment that is homologous with the centric region of at least one major chromosome.

Intercalary deletions, one in each arm, though possible, seem unlikely as a source of fragment chromosomes, since the infrequency of spontaneous breaks and the absence of intercalary losses (8) make such an event, involving four separate breaks, of considerable rarity. A more plausible explanation can be derived from a consideration of overlapping inversions. The presence of an acentric ring chromosome in meiosis of *Tradescantia* indicates the presence of either overlapping or adjacent inversions (14). If of the former type, figure 6 illustrates the manner by which an arm of a chromosome can be shortened by the transfer of chromatin to its homologue. Crossing-over in region *B* produces greatly abbreviated and greatly lengthened arms, *ABG* and *ADCFEBCDEFG*. Arm *ABG* has the terminal and centric regions intact, and pairing could then take place between it and its progenitor chromosome. The other arm could be lost in a similar manner or in other ways.

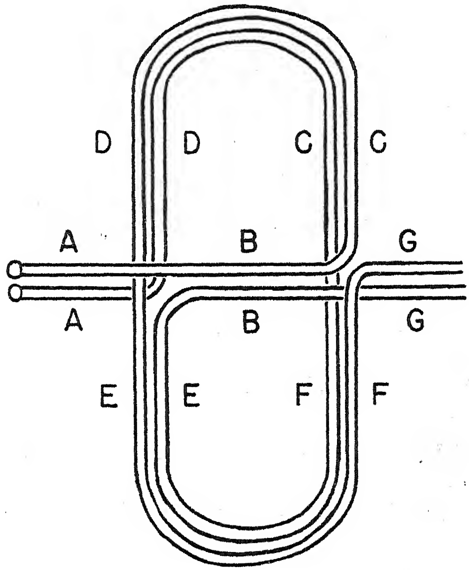


FIG. 6.—Diagrammatic representation of pairing in overlapping inversions. Cross-over in region *B* leads to greatly altered chromatids.

Reciprocal translocations suggest the simplest means of fragment production. An unequal translocation, involving breaks near the centromere of one chromosome and the distal end of another, would yield a chromosome with a subterminal centromere that would be viable, since no loss of chromatin results. This could be carried on until chance provided the opportunity for similar translocation in the other arm. If the material in the fragment were largely heterochromatin, as the genetic evidence seems to bear out, then this provides a method

not only for the origin of fragments but also for a reduction in chromosome number with no essential loss of chromatin.

Since in nuclei with two fragments both have not been seen to pair simultaneously with different chromosomes or with both ends of the same chromosome, it seems probable that while one end of the fragment possesses terminal homologies with the other chromosomes, the other end does not. The explanations then apply only to one arm, and until further data are obtained, they should be considered only in the light of suggestions.

WHITAKER (17) considers it very likely that the fragment arose in a polyploid species of *Tradescantia* because of the greater survival value aneuploids would have where the genom is duplicated. The present phylogenetic knowledge of the genus makes the reverse appear more plausible. Ample phyto-geographical and cyto-taxonomic evidence has shown that most, if not all, of the tetraploid *Tradescantia* have arisen from their diploid progenitors through autopolyploidy (1, 2), and since the diploids possess the fragment it is probable that the fragments originated in them before they gave rise to the tetraploids.

Summary

A study of 800 fragment chromosomes at metaphase in the pollen tubes of *Tradescantia* revealed that somewhat less than one-half of their number show secondary association with the major chromosomes, pairing taking place at either the ends of the chromosomes or at the centric regions. Judging from these pairing relationships, the fragment, which possesses a median centromere, has the end of one arm homologous with the distal portion of one of the heterobrachial major chromosomes, while the centric region is probably homologous with a similar region in the same or another of the heterobrachial chromosomes. The end pairing is apparently specific; the centric pairing may or may not be. The possible origin of one arm of the fragment is discussed; nothing much can be stated as to the structural makeup of the other arm of the fragment.

AGRICULTURAL EXPERIMENT STATION
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LITERATURE CITED

1. ANDERSON, E., and SAX, K., A cytological monograph of the American species of *Tradescantia*. BOT. GAZ. 97:433–475. 1936.
2. ANDERSON, E., and WOODSON, R. E., The species of *Tradescantia* indigenous to the United States. Contr. Arnold Arbor. No. 9. 1935.
3. DARLINGTON, C. D., Studies in *Prunus*. I, II. Jour. Genet. 19:213–256. 1928.
4. ———, Chromosome behaviour and structural hybridity in the Tradescantiae. Jour. Genet. 21:207–286. 1929.
5. ———, Chromosome behaviour and structural hybridity in the Tradescantiae. II. Jour. Genet. 35:259–280. 1937.
6. ———, Recent advances in cytology. 2d ed. Blakiston, Philadelphia. 1937.
7. ———, Misdivision and the genetics of the centromere. Jour. Genet. 37:341–364. 1939.
8. GILES, N. H., Spontaneous chromosome aberrations in *Tradescantia*. Genetics 25:69–87. 1940.
9. ———, Chromosome behavior at meiosis in triploid *Tradescantia* hybrids. Bull. Torr. Bot. Club 68:207–221. 1941.
10. LAWRENCE, W. J. C., The genetics and cytology of *Dahlia variabilis*. Jour. Genet. 24:257–306. 1931.
11. METZ, C. W., Chromosome studies on the Diptera. II. The paired association of chromosomes in the Diptera and its significance. Jour. Exp. Zoöl. 21:213–262. 1916.
12. RHOADES, M. M., Studies of a telocentric chromosome in maize with reference to the stability of its centromere. Genetics 25:483–520. 1940.
13. STURTEVANT, A. H., and DOBSHANSKY, T., Reciprocal translocations in *Drosophila* and their bearing on *Oenothera* cytology and genetics. Proc. Nat. Acad. Sci. 16:533–536. 1930.
14. SWANSON, C. P., The distribution of inversions in *Tradescantia*. Genetics 25:438–465. 1940.
15. ———, The use of acenaphthene in the pollen tube technique. Stain Technol. 15:49–52. 1940.
16. UPCOTT, M. B., The parents and progeny of *Aesculus carnea*. Jour. Genet. 33:135–150. 1936.
17. WHITAKER, T. W., Fragmentation in *Tradescantia*. Amer. Jour. Bot. 23:517–519. 1936.

PROVITAMIN A AND VITAMIN C IN THE GENUS LYCOPERSICON[1]

RALPH E. LINCOLN, F. P. ZSCHEILE, J. W. PORTER, GEORGE W. KOHLER, AND RALPH M. CALDWELL

Tomatoes are a good source of both provitamin A (β-carotene) and vitamin C, and they are of greater value in human nutrition as a source of essential vitamins and minerals than as a source of energy. Studies here reported were made to determine the genetic variability in the contents of provitamin A and vitamin C of commercial varieties and related forms and species of *Lycopersicon*.

According to MULLER (3), the genus *Lycopersicon* includes six species, five of which have been introduced into the United States by the Division of Plant Exploration and Introduction, U.S. Department of Agriculture. The species are widely divergent in fruit, vegetative, and growth characteristics, and it is reasonable to expect that considerable differences may exist also in their contents of provitamin A and vitamin C. The experiments were conducted at Lafayette during the summer of 1942. At least five plants of each strain were grown, and usually a sample of fruit was collected for analysis from a single plant that appeared typical of that strain. Samples of fruit for analysis were collected from August 18 to September 28. A control variety, Baltimore (Indiana strain), was planted every fifth row and was analyzed at intervals throughout the season. This variety served as a control on variation in vitamin content arising from environmental differences in the plot and due to different dates of sampling.

Carotenoid pigments were extracted with an acetone-hexane mixture. Carotenols (5) were removed by successive extractions with 6 per cent aqueous diacetone-alcohol, 90 per cent methanol, and methanolic KOH. Spectroscopic observations on the solution of carotenes were made for the estimation of lycopene. The β-carotene fraction was separated from the lycopene fraction on a MgO column, and measurements of absorption were made at several wave-lengths for the determination of the former pigment.

In the analysis for vitamin C, the extract was prepared according to MORELL'S procedure (2) for plant materials, and an aliquot of the metaphosphoric-acid extract was titrated with sodium 2, 6-dichlorobenzenoneindophenol (1).

The ranges of β-carotene, lycopene, and vitamin C contents, and percentage dry matter of the fruits in the various species and strains are given in table 1.

The content of β-carotene does not appear to be a distinct species characteristic, although *L. peruvianum* has a much lower average content than the other species analyzed. The content of β-carotene varied most widely in a segregating population from the cross *L. hirsutum* × *L. esculentum*, back crossed to *L. esculentum*. Since *L. hirsutum* did not fruit in the field, no carotene analyses have been made of this species, and it has been impossible to determine whether this parent is responsible for the high carotene content or whether it is due to new gene combinations. The latter possibility seems reasonable, since *L. hirsutum* has "green" fruits as does *L. peruvianum*, and the commercial parent has been found to have a low β-carotene content. The extreme variation in lycopene content may be noted in the table. Although lycopene has no known nutritional value, the red-

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ness of the fruit appears to be related to its lycopene content and is of interest because of consumer demand.

The commercial strains of *L. esculentum* are reasonably uniform for a low content of vitamin C as compared with either *L. pimpinellifolium* or *L. peruvianum*. Since the characters of the commercial tomato are derived almost entirely from *L. esculentum*, this is not surprising. REYNARD and KANAPAUX (4) reported a similar range in content of vitamin C in these species, except that *L. peruvianum* was found to have a range only slightly greater than that of *L. pimpinellifolium*.

The variation in genetically diverse material was much greater than that in the control variety, Baltimore, which could be ascribed to soil variability or date of sampling.

This investigation has shown that the strain highest in β-carotene contained nine times as much as the highest commercial variety. The strain highest in vitamin C contained three times as much vitamin C as the highest commercial variety. These high strains contained approximately thirteen times as much β-carotene and four times as much vitamin C as the average content of the most widely grown commercial varieties.

These strains are of little commercial value without further development but are valuable for use as parents in a program to develop high vitamin varieties of commercial tomatoes. How rapidly such varieties may be developed will depend to a great extent upon the genetic complexity of the inheritance of vitamin content. It appears unlikely that the maximum variation in vitamin content has been found.

These investigations are being continued toward the object of developing varieties of high vitamin contents.

DEPARTMENTS OF BOTANY AND AGRICULTURAL CHEMISTRY
PURDUE UNIVERSITY AGRICULTURAL EXPERIMENT STATION
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TABLE 1

RANGES OF CAROTENE, LYCOPENE, VITAMIN C, AND DRY-MATTER CONTENTS IN LYCOPERSICON SPECIES AND CROSSES

(FRESH-WEIGHT BASIS)

SPECIES OR CROSS	NO. OF STRAINS ANALYZED FOR		β-CAROTENE FRACTION (MICROGRAMS/GM.)	LYCOPENE (MICROGRAMS/GM.)	VITAMIN C (MICROGRAMS/GM.)	DRY MATTER (%)
	Carotenoids	Vitamin C				
Red- and yellow-fruited species						
L. esculentum						
Commercial varieties....	23	31	2.1– 8.0	32 –111	104– 446	4.0– 8.0
Baltimore (Indiana strain)............	*	†	4.9– 7.9	44 –111	193– 316	4.5– 6.2
Foreign strains.........	108	161	1.0–19.1	0.8–187	143– 735	4.0–13.4
L. pimpinellifolium........	25	30	6.6–19.2	37 –463	401– 865	5.9–16.8
Green-fruited species						
L. peruvianum...........	18	18	0.7– 3.6	None–1.4	386–1194	8.3–14.8
Cross of red-×green-fruited species						
[F_1(L. esculentum×L. hirsutum)×L. esculentum]..	86	107	0.6–67.5	None–115	84– 534	3.4–10.7

* Four observations. † Six observations.

LITERATURE CITED

1. BESSEY, O. A., and KING, C. G., The distribution of vitamin C in plant and animal tissues, and its determination. Jour. Biol. Chem. 103:687–698. 1933.
2. MORELL, S. A., Rapid photometric determination of ascorbic acid in plant materials. Ind. Eng. Chem. Anal. Ed. 13:793–794. 1941.
3. MULLER, C. H., A revision of the genus *Lycopersicon*. U.S.D.A. Misc. Publ. 382. 1940.
4. REYNARD, G. B., and KANAPAUX, M. B., Ascorbic acid (vitamin C) content of some tomato varieties and species. Proc. Amer. Soc. Hort. Sci. 41: 298–300. 1942.
5. ZSCHEILE, F. P., Plastid pigments, with special reference to their physical and photochemical properties and to analytical methods. Bot. Rev. 7:587–648. 1941.

MAINTAINING THE SURFACE OF HONES

Hones that have become hollowed and scratched can be reconditioned and maintained in optimum condition by the following procedure.

Hollows and scratches are ground off with abrasive paper or cloth having particles of fine texture. Hones with relatively deep hollows can be flat-surfaced in less than 15 minutes through the use of an electric wood-sanding machine. Padding should be removed from the backstop to provide a firm flat surface against the back of the abrasive belt. When a machine is not available, the hone is rubbed against an abrasive sheet fastened to a flat surface. This method is quite satisfactory, particularly if the hone is reconditioned before the surface has become too worn.

Striations produced by the grinding can be removed by rubbing together two flat-surfaced hones. This operation can be shortened by the use of a very fine abrasive, and the grit produced during this polishing should be continuously washed away. When running water is not available, washing in still water should be frequent. Polished surfaces can be readily achieved by rubbing together two yellow Belgian hones or a yellow Belgian and a German blue-water hone; more time is required to finish the surfaces when two blue hones are rubbed together, since then there is a greater tendency toward scratches.

Hone surfaces can be maintained in optimum condition by such reconditioning after each use. The grinding operation need be repeated only when it becomes evident that polishing for a reasonable time is insufficient to keep the surfaces flat.—MARTIN HAWRYLOW, *New York State College of Forestry, Syracuse, New York.*

MORPHOLOGICAL AND CYTOLOGICAL STUDIES ON CARICA PAPAYA

LOIS THOMSON FOSTER

Carica papaya, native to tropical America, where the fruit is an important article of diet, is now cultivated extensively in all tropical regions, as well as in Texas and Florida. Papain, a digestive enzyme, is obtained from the latex of this plant. The papaya plant is a semi-woody tree which grows to a height of 10–25 feet.

Although considerable work of a physiological nature has been reported, the morphological and cytological work concerning *C. papaya* seems to have been rather incomplete, and accounts differ as to the development of the megagametophyte and embryo in this particular species. USTERI (10) reported that the megagametophyte was of the normal type, with the antipodals disappearing soon after their formation. He observed the development of an axial row but stated that the micropylar megaspore was the functional one. Although KRATZER (3) confirmed the report of a normal type of megagametophyte development, he was undecided as to the number of cells composing the axial row and claimed that either the chalazal or the micropylar megaspore might become functional. The pollen tube was described by KRATZER as a large structure which, as it enters the nucellus, becomes somewhat arched and forms small pouches. KRATZER compared the pollen tube in *C. papaya* with that found in some of the Cucurbitaceae by KIRKWOOD (2).

Contrary to the views of the earlier workers, HEILBORN (1) reported that the nucleus of the megaspore mother cell divides to form a binucleate embryo sac and that no tetrad of spores is produced, although reduction division occurs. The five-nucleate mature megagametophyte observed by him was classified as a reduced *Lilium* type or as a modification of the *Plumbagella* type. Few observations have been made concerning embryo development in this species.

SCHAFFNER (4), working with pistillate plants in the greenhouse, induced nearly mature fruits to develop without pollination through reduction of the transpirational surface of the plants.

MATERIAL AND METHODS.—The plants were grown in the greenhouse at the University of Wisconsin from seed obtained in Florida. Although both hermaphroditic flowers and dioecious types are formed, this investigation was limited to a study of pistillate and staminate plants. The fruits of these forms are characterized by a more spherical or ovoidal shape in comparison with the elongated parthenocarpic fruits.

Pistillate flowers were hand-pollinated by slightly untwisting the petals of a partially opened flower and dusting the pollen on to the stigma (9). Following pollination, measurements of the width and length of the pistils were made at intervals of 2–3 days.

A series of pistillate flowers and pistils at various stages of development before and after pollination were removed and fixed in F.A.A. or Karpechenko's modification of Navashin's solution, after being dipped into Carnoy's solution. The material was then carried through a butyl-alcohol series for dehydration and imbedded in paraffin. Sections were cut at 10–12 μ. Material was stained in safranin and fast green, in Chlorazol Black E, but best results were obtained by the use of Delafield's haematoxylin diluted to one part of haematoxylin to three parts of distilled water.

Pollen grains were germinated artificially, according to the method described

by TRAUB and O'RORK (7). The pollen was sprinkled on to slides covered with a thin layer of agar sucrose medium.

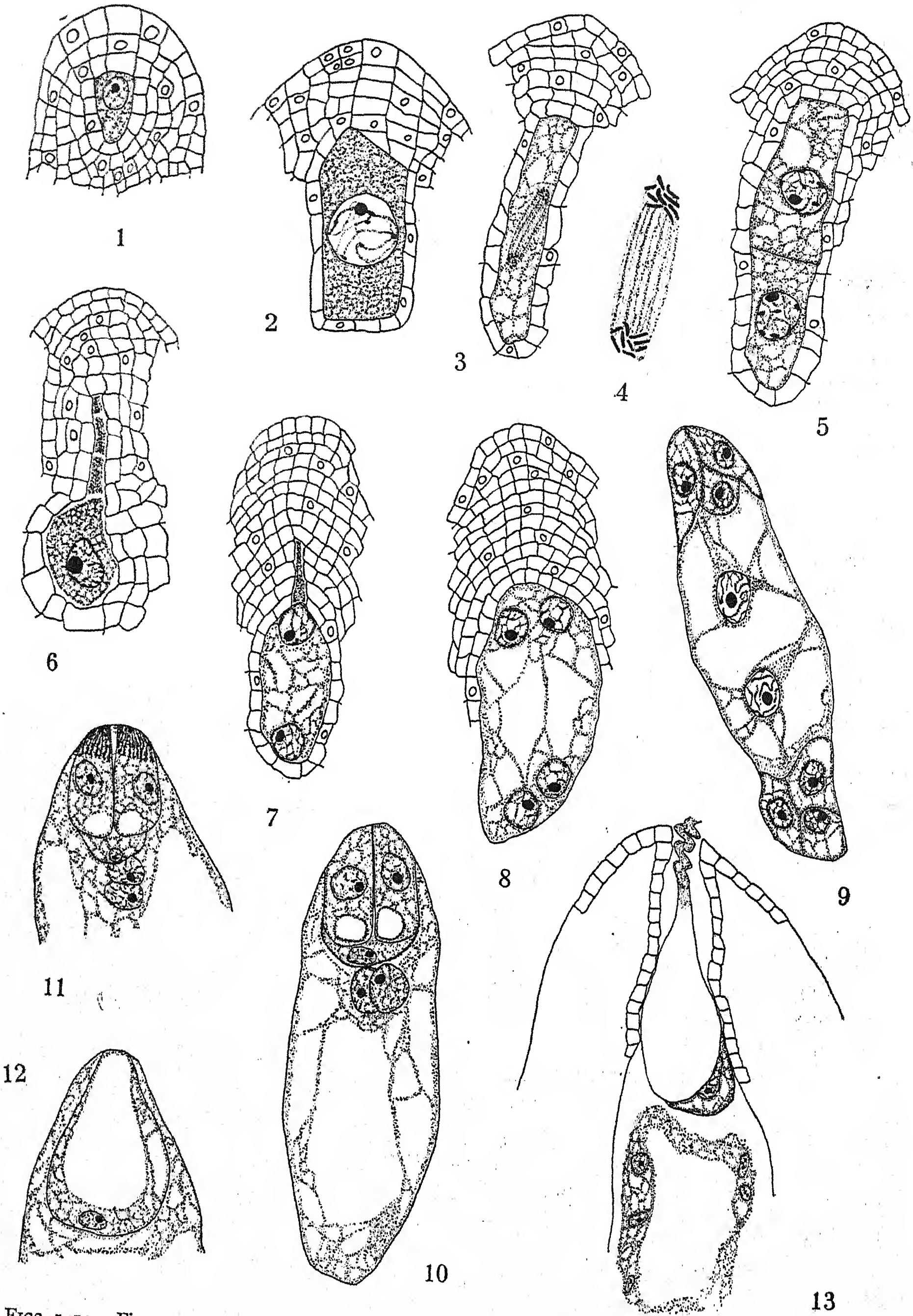
Observations

The nucellus of the young ovule appears as a slight enlargement at the end of an unusually long stalk (fig. 27), and the primordium of the outer integument seems to develop prior to the appearance of the inner integument (fig. 28), as stated also by KRATZER (3). As the inner integument begins to differentiate, the primary sporogenous cell can be seen (fig. 1) imbedded in three layers of cells, the two innermost apparently comprising the parietal tissue. The ovule is somewhat curved and the two integuments differentiate when the megaspore mother cell appears imbedded in about five layers of nucellar tissue (fig. 2). At heterotypic division, the haploid number of chromosomes is nine (figs. 3, 4). This agrees with the chromosome count by SUGIURA (6). An axial row of four cells is present, and the chalazal megaspore becomes the functional one (figs. 5, 6). These observations are contrary to the findings of USTERI (10), who claimed that the micropylar megaspore becomes functional, and HEILBORN (1), who stated that no axial row is formed.

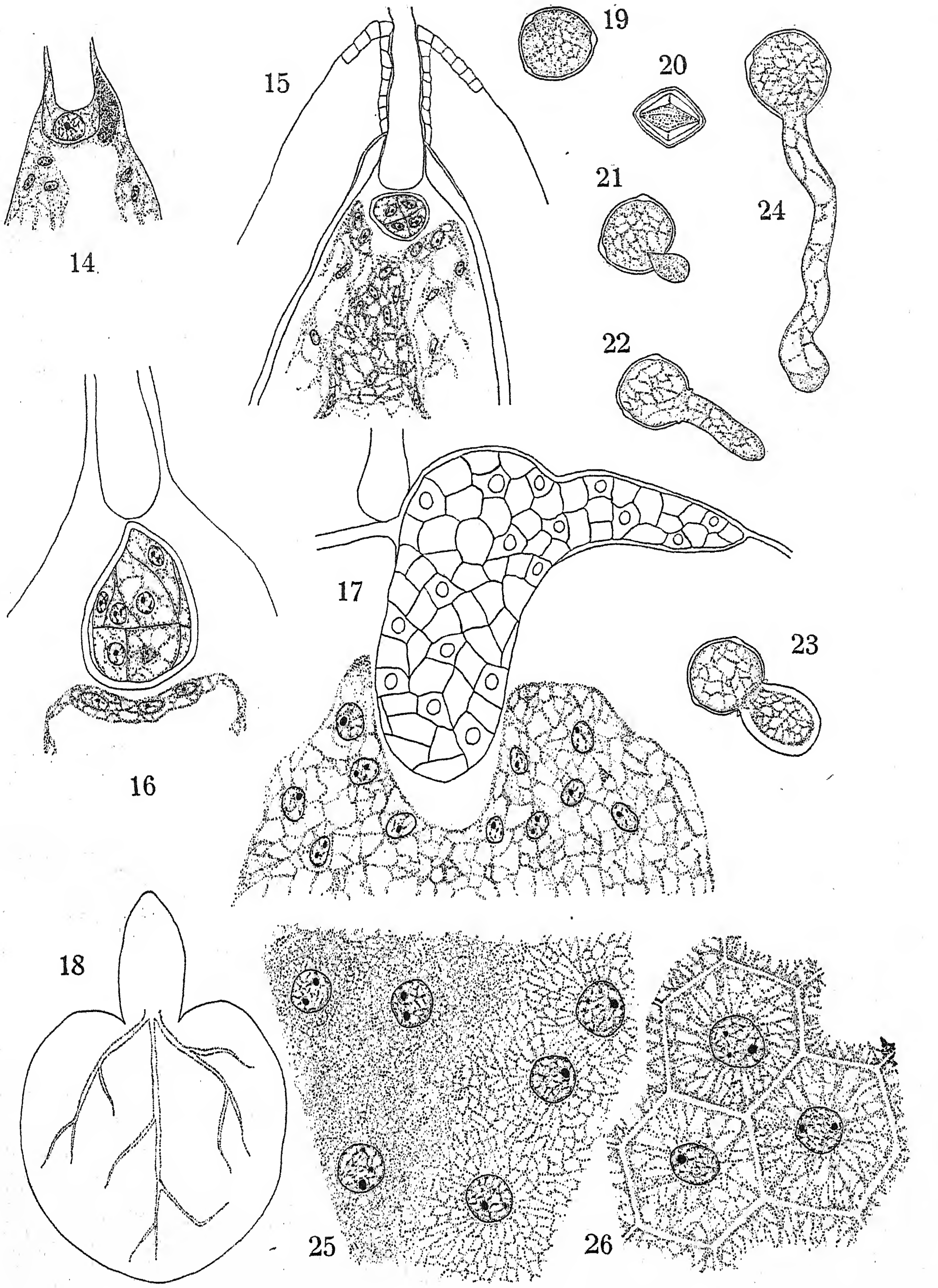
The functional megaspore divides to form a two-nucleate megagametophyte followed by a four-nucleate stage (figs. 7, 8). A seven-celled, eight-nucleate gametophyte of the normal type is formed (fig. 9). This confirms the work of USTERI (10) and KRATZER (3), but is contrary to the findings of HEILBORN (1). The antipodal cells disintegrate almost immediately after their formation and well before pollination occurs. The egg apparatus appears to be extremely minute in comparison with the large embryo sac and massive ovule in which it is located (figs. 10, 30). In the young seven-celled gametophyte, the synergids are about the same size as the egg and are characterized by nuclei at their micropylar ends and conspicuous vacuoles at their free ends. A filiform apparatus develops late in the differentiation of the synergids (fig. 11). The egg cell enlarges considerably at this stage (fig. 12) and contains a large vacuole at the micropylar end. Various stages in megagametophyte development, from two-nucleate to young seven-celled, eight-nucleate forms, are found within different ovules of the same pistil. It is evident that all the thousands of ovules which may be present at a given time within a single pistil are not at the same stage of development.

Under greenhouse conditions the first evidences of pollen tubes within the ovules are found in pistils 10 days after pollination, as compared with the experiments of TRAUB and O'RORK (8), who state that the pollen tubes reach the ovules in the apical end of the pistil within a day and a half and those in the proximal end within 5 days after pollination. The pollen tubes are large massive structures which usually take a rather uniform dark purple stain with Delafield's haematoxylin. A tube grows through the micropyle and becomes slightly arched as it enters the nucellus. The cytoplasmic content seems more granular in some of the tubes as they grow through the micropyle, but no differentiation was observed in the contents of the ends of the older tubes which have reached the nucellus and the gametophyte (fig. 13).

The size of pollen tubes found within the ovules compares favorably with that of the tubes of artificially germinated pollen grains. Germination occurred within one hour after the grains had been placed on an agar-sucrose medium. The tubes, which are unusually large and bounded by relatively heavy membranes, typically become bulbous at the tips (figs. 19–



FIGS. 1–13.—Fig. 1, young nucellus showing primary sporogenous cell and parietal tissue Fig. 2, megaspore mother cell; prophase. Fig. 3, same; heterotypic anaphase. Fig. 4, heterotypic anaphase; enlarged spindle from fig. 3. Fig. 5, interkinesis. Fig. 6, functional chalazal macrospore with three disintegrating megaspores. Fig. 7, two-nucleate megagametophyte; disintegrating megaspores. Fig. 8, four-nucleate megagametophyte. Fig. 9, young seven-celled eight-nucleate megagametophyte. Fig. 10, mature megagametophyte after disappearance of antipodal cells. Fig. 11, mature megagametophyte showing filiform apparatus of synergids. Fig. 12, egg cell of mature megagametophyte from fig. 10. Fig. 13, twisted pollen tube with bulbous tip in contact with young zygote, 16 days after pollination; several endosperm nuclei present.



FIGS. 14–26.—Fig 14, zygote 18 days after pollination showing pollen tube, endosperm nuclei, and disintegrating synergid. Fig. 15, embryo 23 days after pollination showing surrounding sheath; persistent pollen tube and abundant free-nuclear endosperm. Fig. 16, embryo with surrounding sheath 35 days after pollination; persisting pollen tube and free-nuclear endosperm. Fig. 17, many-celled embryo about 64 days after pollination showing sheath, haustorial suspensor, persistent pollen tube, and abundant free-nuclear endosperm. Fig. 18, nearly mature embryo 4 months after pollination showing provascular tissue in cotyledon. Fig. 19, viable pollen grain. Fig. 20, shriveled pollen grain. Figs. 21–24, stages in germination of pollen grains showing bulbous ends of pollen tubes. Fig. 25, beginning of transition from free-nuclear to cellular endosperm, approximately 79 days after pollination. Fig. 26, cellular endosperm.

24). Germination was estimated to be 85–90 per cent.

Fertilization occurred at approximately 13–15 days after pollination. Before fertilization the egg nucleus is quite small, compared with the nuclei of the other cells comprising the mature gametophyte (figs. 10, 11). After fertilization the zygote enlarges considerably and persists for about 5–8 days (figs. 13, 14). There occurs a comparatively great increase in the growth of the fruit during the development of the zygote (table 1). At this stage the end of the conspicuously swollen pollen tube can be seen close to the wall of the zygote and is still traceable back through the nucellus and integumentary region. Some portions of the disintegrating synergids still remain. A few endosperm nuclei are formed, with a comparatively greater number present in the immediate vicinity of the zygote. However, no part of the endosperm is in direct contact with the zygote, which seems to be surrounded by a conspicuous envelope.

There is a definite lag in the development of the embryo, since four-celled embryos do not appear until 23–28 days after pollination (fig. 15). Early divisions occur irregularly and the endosperm grows much more rapidly than the embryo. Ovules approximately 32–35 days after pollination contain embryos with about eight to sixteen cells (fig. 16). Pollen tubes are still persistent at these stages, and all the embryos observed are surrounded by a sheath (figs. 15, 16). The endosperm tissue, still in a free-nuclear condition (although it has increased considerably in amount), is apparently not in direct contact with the embryo.

At about 64 days after pollination there has developed a many-celled, peculiarly shaped embryo which possesses a suspensor-like projection extending off at one side (fig. 17), which appears to be haustorial in nature. The pollen tube still persists at this stage, with its tip closely pressed against the embryo, and it can still be traced back through the integumentary region. The bulbous tip of the

TABLE 1

CORRELATION OF SIZES AND AGES OF FRUITS WITH INTERNAL DEVELOPMENT

Length and width (cm.)	Age	Internal development
	Before pollination:	
1.5 × 0.75	5–7 days	Megaspore mother cell, megasporogenesis, functional megaspore; provascular tissue in funiculus and outer integument of ovule
2.2 × 1.2	2 days	All stages in megagametophyte development; vascular differentiation in funiculus and outer integument
	After pollination:	
2.7 × 1.3	3 days	Mature megagametophyte
3.4 × 1.5	6 days	Mature megagametophyte
3.7 × 1.9	10 days	Pollen tubes entering ovules; well-differentiated vascular tissue in funiculus and outer and inner integuments
3.8 × 1.9	13–15 days	Fertilization
4.0–4.2 × 2.1–2.15	16–19 days	Zygote; free-nuclear endosperm
4.5 × 2.4	23–30 days	Four-celled embryo; free-nuclear endosperm; elongated nucellar cells in chalazal region of ovule
4.7–5.0 × 2.45–2.5	32–35 days	Eight-celled embryo; free-nuclear endosperm
9.5 × 6.3	64 days	Advanced embryo development and abundant free-nuclear endosperm; pollen tube persisting
12.0 × 7.5	79 days	Parts of embryo differentiated; transition from free-nuclear to cellular endosperm
14.2 × 7.9	4 months	Nearly mature embryo; cellular endosperm

pollen tube is similar to those observed by KRATZER (3) and KIRKWOOD (2) in some of the Cucurbitaceae, although structures analogous to the pouches described by these workers were not observed in the pollen tube of *C. papaya*. KIRKWOOD stated that the nutrition of the embryo in some of the Cucurbitaceae was brought about by means of a haustorial pollen tube which obtained its nourishment from the vascular tissue. The abundant endosperm is still free-nuclear, and the embryo appears very minute in comparison with the amount of endosperm present (fig. 33). At this stage the embryo also shows the characteristic film surrounding its upper portion.

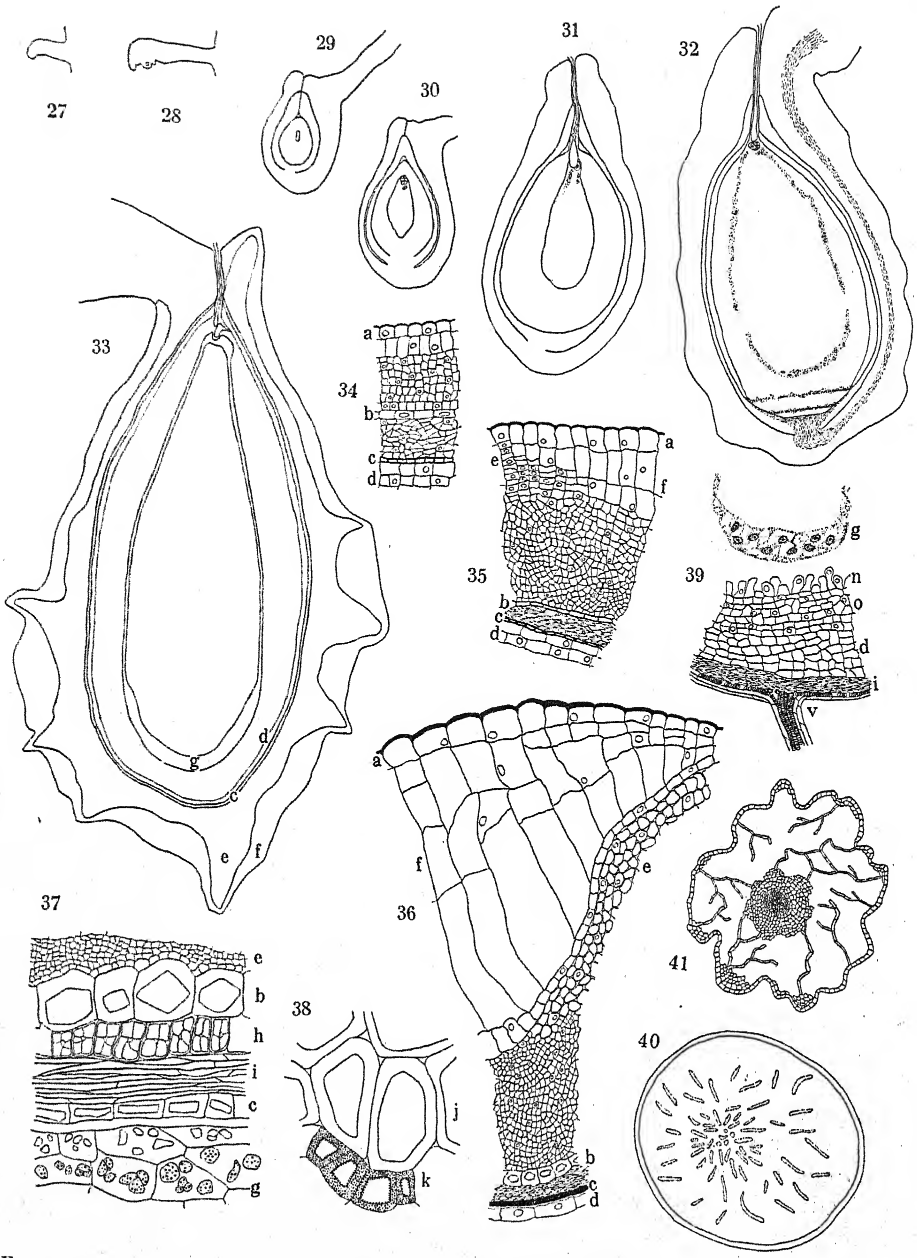
The persistence and position of the pollen tube, the lag in development of the embryo, and the separation of the young embryo from the endosperm, together with the presence of a suspensor, might suggest an unusual nutritive relationship. Although more work is needed to determine the exact interrelationships, it might be suggested that the persistent pollen tube may be closely associated with the nutrition of the embryo in its early stages of development. As the embryo grows older and is more imbedded in the endosperm, there then may occur a shift in the nutritive arrangement and the endosperm might become more functional in relation to the embryo.

Approximately 11–12 weeks after pollination the endosperm shows a transition from the free-nuclear state to a cellular condition (figs. 25, 26). This change occurs toward the micropylar end of the ovule first and proceeds to the chalazal end. The cytoplasm becomes less dense just before cell formation, and numerous minute vacuoles appear within it. After the cell membranes have been laid down, strands of cytoplasm seem to extend from one cell to the next, suggesting division by cell plate formation.

The embryos contained in ovules at 11–12 weeks after pollination have assumed the shape but not the size of the embryo found in the mature seed (fig. 18). The freshly dissected embryos at 4 months after pollination show two broad flattened cotyledons and a comparatively small hypocotyl. The provascular strands in the cotyledons are well differentiated. At this stage the endosperm, which is completely cellular, contains an abundance of aleurone grains and oil (fig. 37), but starch is absent.

Only a very small percentage of the ovules in a given pistil produced embryos in the material studied. This lack of embryo development cannot be due in every case to insufficient pollen, since many ovules which did not develop embryos contain abundant 3-n endosperm tissue, and pollen tubes are also present. In a few instances there is evidence of disintegration of the egg and synergids, even when endosperm nuclei are present. Enzymatic action of the papain present in the ovary wall apparently causes the queer "digested" appearance occasionally seen in the outer tissues of the ovule. Latex tubes are found in abundance in the ovary wall but not in the ovules. In the younger fruits they appear cellular, but in more mature fruits the cross walls apparently disintegrate, leaving long branched tubes. It is very likely that the latex tubes bear some relationship to the vascular system, since they are often located near vascular bundles.

The development of the seed coat was studied in relation to the internal changes observed within the ovules. The seed coat arises chiefly from the tissue of the outer integument, which greatly increases in size in comparison with the inner integument. The inner integument also persists as a distinct layer in the mature seed. During its rapid growth the outer integument becomes differentiated



FIGS. 27–41.—Fig. 27, very young ovule showing nucellus and conspicuous stalk. Fig. 28, section of young ovule at development of primary sporogenous cell; outer integument developing earlier than inner. Fig. 29, anatropous ovule about 2 days before pollination showing massive funiculus, inner and outer integuments, and young megagametophyte. Fig. 30, section of ovule 3 days after pollination containing egg apparatus within large megagametophyte. Fig. 31, ovule 16 days after pollination showing considerable enlargement of outer integument and nucellus; pollen tube, young zygote, and a few endosperm nuclei. Fig 32, ovule 23 days after pollination showing small embryo, persistent pollen tube, abundant free-nuclear

into two distinct parts, the outer layer or sarcotesta and the inner portion or endotesta, as named by STEPHENS (5). The endotesta appears as a compact tissue, which at various intervals forms ridges parallel to the long axis of the seed. The gelatinous transparent sarcotesta, which is composed of large cells, forms the outer portion of the seed coat.

Just before pollination, the two integuments each consist of four or five layers of parenchyma (fig. 29). Three days after pollination, before pollen tubes were observed in the ovules, the outer integument increases slightly in width by means of cell division. There is a slight thickening of the outer walls of the outer epidermis of this integument, and the cells of the inner epidermis of the inner integument show evidence of the deposition of some material, stated by STEPHENS (5) to be tannin (fig. 30).

About 16–19 days after pollination, corresponding to the time of zygote development, all the cells of the inner integument, with the exception of the inner epidermal layer, start to elongate transversely (fig. 34). Further thickening of the walls of the outer epidermal cells of the outer integument is evident (fig. 34*a*), and the outer integument and nucellus are increasing in size through cell division (figs. 31, 34).

When young embryos are present within the ovules, approximately 23–30 days after pollination, the inner integument (except for its inner epidermis) appears as a compact layer between the outer integument and the nucellus. The increase in thickness of the outer integument at this time is due chiefly to the action of meristematic bands, which appear under the outer epidermis and mark the future position of the endotestal ridges (figs. 32, 35*e*). The epidermal and hypodermal cells between the meristematic regions which divide and increase greatly in size (fig. 35*f*) will later become the sarcotesta.

As the embryo attains a later stage of development, about 64 days after pollination, the endotesta and sarcotesta of the outer integument are clearly discernible (fig. 33). The cells of the endotestal ridges have increased greatly in number but are only slightly enlarged (figs. 33,

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endosperm, and vascular strand in outer integument branching into inner integument at chalazal end; irregular endotestal ridges becoming evident in outer integument. Fig. 33, section of ovule 64 days after pollination, still containing comparatively small embryo and persistent pollen tube: *c*, inner integument; *d*, nucellus; *e*, endotesta; *f*, sarcotesta; *g*, abundant free-nuclear endosperm. Fig. 34, section of seed coat 16 days after pollination: *a*, outer epidermis of outer integument; *b*, inner epidermis of outer integument; *c*, inner epidermis of inner integument showing deposition of tannin; *d*, nucellar tissue. Fig. 35, section of seed coat 23 days after pollination. Outer integument: *a*, outer epidermis; *b*, inner epidermis; *e*, meristematic cells later forming endotestal ridges; *f*, large cells resulting from division and enlargement of epidermal and subepidermal cells between meristematic regions, and later becoming sarcotesta. Inner integument: *c*, inner epidermis; *d*, nucellus. Fig. 36, section through seed coat 64 days after pollination. Outer integument: *a*, outer epidermis with greatly thickened outer walls; *b*, inner epidermis; *e*, endotestal ridge; *f*, sarcotesta. Inner integument: *c*, inner cutinized epidermis; *d*, only remaining layer of enlarged nucellar cells. Fig. 37, enlarged portion of mature seed coat. Outer integument: *e*, small thin-walled compact cells; *b*, inner epidermis with greatly enlarged cells and conspicuous crystals. Inner integument: *h*, thick-walled stone cells apparently originating from outer epidermis; *i*, enlarged elongated cells composing great part of inner integument; *c*, inner cutinized epidermis containing tannin; *g*, cellular endosperm containing aleurone grains. Fig. 38, portion of endotestal ridge of mature seed: *j*, greatly enlarged cells; *k*, somewhat smaller cells with pitted walls. Fig. 39, section of nucellus and inner integument at chalazal end of ovule 64 days after pollination: *g*, free-nuclear endosperm; *n*, elongated nucellar cells; *o*, nutritive region of nucellus; *d*, hyaline region of nucellus; *i*, inner integument; *v*, vascular strand. Fig. 40, cross section through chalazal region of inner integument 4 months after pollination showing umbrella-like arrangement of vascular elements. Fig. 41, cross section of spongy funiculus 79 days after pollination showing amphicribral vascular bundle.

36). Those cells adjacent to the inner epidermis of the outer integument are small, while those composing each ridge appear progressively larger toward the outermost portion of the ridge (fig. 36*e*). Most of the endotestal ridges lie parallel to the longitudinal axis of the seed. There also occurs a slight increase in the number and a great increase in the size of the epidermal and hypodermal cells located between the ridges (fig. 36*f*), so that the sarcotesta appears transparent and gelatinous in fresh material. Owing to the presence of this layer, the surface of the seed is smooth while it remains within the fruit. The outer walls of the outer epidermal cells become greatly thickened (fig. 36*a*). The inner epidermal cells of the outer integument increase in size, their walls become thickened, and a calcium oxalate crystal is formed in each cell (fig. 36*b*). The inner epidermis of the inner integument is characterized by cells containing an abundance of tannin (fig. 36*c*). All nucellar tissue has apparently disintegrated, with the exception of the outermost layer of enlarged cells adjacent to the inner integument (fig. 36*d*).

In several instances ovules of this age were found, each of which possessed a well-differentiated seed coat without the presence of either embryo or endosperm. Apparently most of the ovules within a pistil develop to a certain extent after pollination, even though fertilization does not occur in some. Changes are seemingly brought about as a result of pollination, which stimulates nucellar and integumentary growth, independent of embryo development.

Although STEPHENS (5) reported the presence of the sarcotesta in the mature seed coat, this region is apparently no longer present in the seed coats of the mature dried seeds studied. The cells composing the tips of the endotestal ridges have been extremely enlarged and possess very thick cell walls (fig. 38*j*), while the innermost region of the endotesta is made up of slightly smaller cells with numerous pits in the walls (fig. 38*k*). The inner epidermal cells of the outer integument still retain their characteristic crystals (fig. 37*b*).

In the inner integument of the mature seed, the outer epidermal layer appears as a row of thick-walled stone cells (fig. 37*h*), and the inner epidermis is composed of greatly enlarged cells containing an abundance of tannin (fig. 37*c*). The other inner integumentary cells are somewhat enlarged and elongated but possess comparatively thin cell walls (fig. 37*i*). These observations concerning the development of the inner integument are contrary to those described by WINTON and WINTON (11), who classify these tissues as an outer layer of longitudinally elongated fibers with thick walls, several rows of transversely elongated fibers, and an inner epidermis of isodiametric cells. The nucellus tissue has completely disappeared, except in the chalazal region.

In tracing the differentiation of the vascular tissue within the ovules, it was found that an ovule at about 6–7 days before pollination contains a single provascular strand which can be traced through the funiculus and the outer integument. However, the ovary wall of a fruit at this age contains well-developed vascular tissue. Some differentiation of the vascular strand into spiral xylem elements and sieve tubes occurs in an ovule approximately 2 days before pollination in the region of the funiculus and outer integument. In more mature ovules, a well-differentiated vascular bundle, amphicribral in type (fig. 41), extends from the funiculus along the endotestal region of the outer integument into the inner integument at the chalazal end, where it

branches widely and irregularly to form an umbrella-like structure (figs. 32, 40).

An interesting specialization of the cells of the nucellar tissue at the chalazal end of ovules is observed 3–12 weeks after pollination. In this region the cells of the nucellus took on the form of rather elongated finger-like processes, unlike the usual nucellus cells (fig. 39*n*). Immediately beneath this modified layer lie two other distinct portions of the nucellus, a dense-staining nutritive region (fig. 39*o*) and a hyaline layer with granular cell contents (fig. 39*d*). Within the inner integument which borders the hyaline region are found the much-branched ends of the vascular strand (fig. 39*v*). Because of the close proximity of the elongated nucellar cells to the endosperm, and also to the vascular strand, some nutritive arrangement is strongly indicated.

Summary

1. In *Carica papaya* the primary sporogenous cell appears within the young ovule as the integuments are beginning to differentiate. One or two layers of parietal tissue are present.

2. The megaspore mother cell is formed simultaneous with the development of the two integuments.

3. Through two meiotic divisions the megaspore mother cell produces an axial row of four megaspores, the chalazal one of which develops into a normal seven-celled, eight-nucleate megagametophyte; the other three megaspores disintegrate. The haploid number of chromosomes is nine.

4. The mature megagametophyte is minute compared with the massive integuments and nucellus. The egg cell enlarges as the megagametophyte grows, and a filiform apparatus develops late in the differentiation of the synergids. The antipodal cells disintegrate almost immediately after their formation.

5. Under greenhouse conditions pollen tubes enter the ovules about 10 days after pollination. Fertilization occurs approximately 13–15 days after pollination. Zygotes persist for about 5–8 days before they divide. Divisions occur irregularly in the young embryos. There is a definite lag in the embryo development of this species, while endosperm development is very rapid, as is differentiation of the seed coat.

6. The large massive pollen tube persists within the embryo sac up to at least 64 days after pollination. The fact that it is closely pressed against the many-celled embryo may suggest a nutritive relationship between it and the young developing embryo.

7. At approximately 79 days after pollination the endosperm shows a transition from the free-nuclear state to a cellular condition, beginning at the micropylar end of the sac. Mature endosperm contains an abundance of oil and aleurone grains, but starch is absent.

8. The seed coat is derived chiefly from the outer integument of the ovule, which grows considerably to form the firm compact endotesta and a transparent gelatinous sarcotesta.

9. The vascular bundle in a more mature ovule extends from the funiculus along the outer integument into the inner integument at the chalazal end of the ovule, where it branches widely to form an umbrella-like structure.

10. Modified elongated nucellar cells at the chalazal end of the ovule, located relatively close to the endosperm and also to the vascular tissue, may play an essential role in some nutritive relationship existing between the entering food supply and the endosperm.

11. Only a very small percentage of the ovules in a given pistil produce embryos, even though endosperm and pollen tubes are present. However, an ovule may grow and develop to a certain extent after pollination, even though fertilization does not occur.

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LITERATURE CITED

1. HEILBORN, O., Taxonomical and cytological studies on cultivated Ecuadorian species of *Carica*. Ark. Bot. **17**:1–16. 1922.
2. KIRKWOOD, J. C., The pollen tube in some of the Cucurbitaceae. Bull. Torr. Bot. Club **33**:327–342. 1906.
3. KRATZER, JOHANNES, Die verwandtschaftlichen Beziehungen der Cucurbitaceen auf Grund ihrer Samenentwicklung. (Mit spezieller Berücksichtigung der Caricaceen, Passifloraceen, Aristolochiaceen, und Loasaceen.) Flora **110**: 275–343. 1918.
4. SCHAFFNER, J. H., Artificial parthenocarpy. Jour. Hered. **26**:261–262. 1935.
5. STEPHENS, E. L., The development of the seed-coat of *Carica papaya*. Ann. Bot. **24**:607–610. 1910.
6. SUGIURA, T., Some observations on the meiosis of the pollen mother cells of *Carica papaya*, *Myrica rubra*, *Aucuba japonica*, and *Beta vulgaris*. Bot. Mag. Tokyo **41**:219–224. 1927.
7. TRAUB, H. P., and O'RORK, C. J., Jr., Papaya pollen germination and storage. Proc. Amer. Soc. Hort. Sci. **34**:18. 1936.
8. ———, and ———, Course of pollen tube growth in *Carica papaya* and *Cucurbita* spp. Nature **143**:562. 1939.
9. TRAUB, H. P., ROBINSON, T. R., and STEVENS, H. E., Papaya production in the United States. U S. Dept. Agr. Circ. no. 633. 1942.
10. USTERI, A., Studien über *Carica papaya* L. Ber. deutsch. Bot. Ges. **25**:485–495. 1907.
11. WINTON, A. L., and WINTON, K. B., The structure and composition of foods. **2**:787–790. 1935.

CURRENT LITERATURE

The Boletaceae of North Carolina. By WILLIAM CHAMBERS COKER and ALMA HOLLAND BEERS. Chapel Hill: University of North Carolina Press, 1943. Pp. viii+93. Illustrated. $7.00.

Despite their relatively large size and conspicuous coloring, the boletes are among the more difficult of the fungi to determine satisfactorily. Aside from spore characters, many of the criteria by which the various species are distinguished are ephemeral and must be observed in fresh specimens. The present volume, with its numerous excellent illustrations, many of them colored, will be of service far beyond the limits of North Carolina, since the great majority of the species discussed are of wide distribution.

The treatment is extremely conservative. All boletes are referred to the three long-established genera—*Boletus*, *Boletinus*, and *Strobilomyces*. No reference is made to recent revisions of the family other than occasional citation of MURRILL'S and SNELL'S names as synonyms, and such citation is far from complete.

There are two keys. In the first, emphasis is placed on features likely to be noted by the experienced collector, with color subordinated; in the second, color dominates. Some species will doubtless lend themselves better to one than to the other; together they should prove more helpful than either alone.

Three new species and four new varieties, all in *Boletus*, are proposed without Latin diagnoses; hence they are technically *nomina nuda*. In addition, there are four new combinations, three in *Boletus* and one in *Boletinus*.—G. W. MARTIN.

The Design of Experiments. By R. A. FISHER. London: Oliver & Boyd, 1942. Pp. xii+236. 12/6 net.

The third edition of this excellent work is only slightly enlarged from the second edition, published in 1937. The two sections which have been added are those numbered 45.1 and 45.2. These deal with more complicated experimental designs and bear the titles: General systems of confounding in powers of 2; and Double confounding. These sections will enable investigators to plan more satisfactory experiments where many factors are involved. The consistent use of this book should enable agricultural workers, particularly, to improve the design of field experiments, in which numerous factors have to be considered. The emphasis upon design is good, especially for those beginning experimental work.—C. A. SHULL.

ERRATA

Professor T. C. CHIN has just informed us that, owing to a misreading of the stage micrometers, some of his data in the Vol. 104, June, 1943, issue, "Cytology of the autotetraploid rye," were incorrectly given and should be changed as follows: P.627, Table 1:

For "No. of stomata per 0.01188 sq. mm." *read* "No. of stomata per 1.846 sq. mm."
For "2.56" *read* "49.35"; *for* "3.14 × 2.18" *read* "44.85 × 62.61" (first line of table).
For "3.26" *read* "55.84"; *for* "3.72 × 2.98" *read* "52.54 × 68.73" (second line of table).

RESPONSES OF ELODEA DENSA TO GROWTH-REGULATING SUBSTANCES

CONTRIBUTIONS FROM THE HULL BOTANICAL LABORATORY 555

LAWRENCE J. KING

Introduction

Numerous studies on *Lemna* by CLARK and co-workers (6), an investigation of *Elodea* by SOLTYS, UMRATH, and UMRATH (23), and several studies on *Chlorella* and other algae, are some of the major investigations with growth-regulating substances using aquatic or submerged plants. The experiments described here are principally concerned with the gross responses of excised shoots and segments of *Elodea densa* when grown in a liquid nutrient medium containing growth substances.

MATERIAL AND METHODS.—Although the generic name *Anacharis* is employed by some writers, since *Elodea* has been proposed as a *nomen conservandum* by Dr. HAROLD St. JOHN (14, p. 292), it is the one adopted in this study. The species of *Elodea* most commonly investigated has been the North American one, *Elodea canadensis* Michx. The species used in this investigation is *Elodea densa* (Planch.) Casp. (verified by C. A. WEATHERBY of the Gray Herbarium). *E. densa* is one of the largest species of the genus and is commonly grown in aquaria. It is South American, with a range from southern Brazil to the delta of the La Plata in Argentina (24).

Using the agar-block technique, HOMÈS and VAN SCHOOR (12) demonstrated that in *Elodea* auxins formed at the growing point inhibited bud growth as well as promoted internodal axial growth. SOLTYS *et al.* (23) studied the responses of various species of the genus to a series of dilutions of l-histidine compounds and indoleacetic acid. VAN OVERBEEK (18) has found auxin in a species of *Elodea*. CORMACK (8) studied the effects of ethylene on root-hair formation in *E. canadensis*.

The most complete account of the anatomy of species of this genus is that of CASPARY (5). Other such studies include the investigations of HOLM (11) and SIDDALL (21). Most of the published work on the anatomy of the species treats of the structure of the shoot apex, stem, and root, and the manner of flower formation. Few investigations have considered the anatomy of the bud-node region of the primary axis and the manner of root formation. CASPARY (5) shows diagrams of the bud node of *Elodea canadensis* but does not describe it in detail. A study of the structure of the bud node has been made here to clarify the responses of these segments to treatment with growth-regulating substances.

In *Elodea densa* the lateral buds are rather regular in their divergence from the primary axis, generally at every twelfth node. Since buds are not formed at every node, the term "bud node" is used to include that portion of the primary axis from which both leaves and a single axillary bud diverge. Excised bud nodes containing a single whorl of leaves, and longer bud-node segments containing three separate whorls, have been employed in the present experiments.

The roots are formed early in the development of the axis and the bud-node region. These roots may be found as small primordia within the cortical tissues of the primary axis, only a few millimeters from the shoot apex. At each bud node there are four dormant root primordia. Two are located in the primary axis in the region of divergence of the secondary axis (one either side of the secondary axis); two are located in the base of the secondary axis near the point of its divergence from the primary axis. CASPARY (5) has termed the undeveloped shoot axis (inclosed within the lateral bud) the secondary axis. These primordia remain inactive throughout the entire length of the shoot. In one shoot about 1 m. in length, a total of eighteen bud nodes was counted. Roots had appeared only at the first bud node at the base of the shoot. Since each of these bud nodes under certain conditions is capable of forming a complete new plant, the potential vegetative reproductive capacity of this species is evident.

In the present experiments the two primordia of the primary axis of the bud node upon emergence are termed the first and second roots; the two primordia of the secondary axis are termed the third and fourth roots. With certain types of treatment fifth roots may appear.

In rooted shoots growing in the culture tanks, generally neither root nor axillary bud growth occurs; the roots develop when a portion of the axis becomes broken or detached. They then develop at the most basal of the nodes of the segment. If the apical bud of the segment is intact, usually none of the axillary buds develop. If the shoot apex is damaged, however, then the bud next below begins to develop. Apical dominance is very evident, even in detached segments containing only two successive buds. In this case the distal bud will grow, while the proximal one will be inhibited and only roots will form.

The light source for most of these experiments consisted principally of 36-inch, 30-watt Mazda fluorescent daylight tubes. The various intensities were all used for a period of 13 hours each day. The intensities in foot candles were measured with a Weston photometer at the rim of the glass tumbler. From data supplied by Dr. ROHRBAUGH (20), the energy value for one of the commonly used intensities, 400 f.c., has been estimated to be 830×10^{-6} gm. cal./cm.^{2}/sec. (since 280 f.c. was equal to 581×10^{-6} gm. cal./cm.^{2}/sec.). ROHRBAUGH's studies (19) include a review of the work on fluorescent light and plant growth.

The chemical name and the source of the principal substances employed are as follows: beta-(indole-3) acetic acid; beta-(indole-3) propionic acid; gamma-(indole-3)-n-butyric acid; alpha naphthalene acetic acid; phenylacetic acid; thiophenyl acetamide; l-tryptophane; glycine; nicotinic acid; ascorbic acid; and l-histidine monohydrochloride—all supplied by Eastman Kodak Co.; thiamin chloride (vitamin B_1) from Merck; alpha naphthalene acetamide from American Chemical Paint Co.; and calcium pantothenate from International Vitamin Corp.

Investigation

EXPERIMENT 1.—Preliminary experiments had demonstrated that excised bud nodes, with a single whorl of leaves and the axillary bud, were capable of developing into complete new plants. Entire shoots and excised bud nodes were found to grow for considerable time in aqueous solutions of growth-regulating substances under fluorescent light or daylight.

Indoleacetic, indolepropionic, indole-

butyric, phenylacetic, and naphthaleneacetic acids; l-tryptophane, and thiamin chloride were the substances used. Stock solutions were prepared by dissolving a small amount of the crystals in hot distilled water (except those substances readily soluble). After repeated heating to near boiling, and with continued shaking, the substances would dissolve. The solutions were then made to volume and stored in a refrigerator at 10° C. Culture solutions were prepared by adding to tap water a sufficient amount of the chemical to make its concentration 1 p.p.m. One series of cultures contained a mixture of vitamin B_1 and indolebutyric acid, each at 1 p.p.m. The slightly alkaline tap water (a recent analysis showed calcium 32 p.p.m., magnesium 11, potassium 1, nitrate 0.1, and sulphate 15) was used as a nutrient medium in most of the experiments. The cultures were not grown under sterile conditions. All experiments with fluorescent light were conducted in a basement room.

Bud nodes were cut from shoots of healthy and firmly rooted plants taken from the stock cultures maintained in tanks in the greenhouse. In cutting out the bud nodes, the size of the stump was determined by making the cut through the internode halfway between the bud node whorl and the next successive whorl. These excised nodes with but a single whorl of leaves measured about 1.0 cm. in length. Since these segments were somewhat variable, all for any given trial were cut at one time and thoroughly mixed, then picked at random and placed in glass tumblers in 200 ml. of solution. In experiment 1 there were placed in each glass three bud nodes and one stem segment (that is, the portion of the axis between two successive bud nodes). The length of such a segment may vary from 7 to 10 cm. It has no buds present, and hence does not usually regenerate. Each compound was tested in triplicate, and controls in tap water alone were tested in replicates of six. The cultures were all grown at an average intensity of about 300 f.c. Readings taken at several different times indicated a temperature range of 28°–30° C. The solutions were not changed during the course of this experiment, although tap water was added to replace that lost through evaporation. The experiment ran August 2–15, 1941.

Detailed observations were made on the cultures every 4 days for 16 days. Data were taken on the length of all roots formed, the number per node, the size of the bud, and general observations on root-hair formation (tables 1, 2). Here, as in the other experiments, the measurements given generally include the sum of the measurements of all the nodes in any one culture. The methods of measuring and tabulating of data in these experiments have been in part adapted from Brown (4). In the two tables the various values of the two series of controls (two series each of three cultures) have been averaged for better comparison with the other cultures. The values in the tables of some experiments show a decrease over values for earlier growth periods. This is chiefly due to death of roots, loss of bud scales, etc.

In the duplicate series of controls in tap water, consisting of eighteen bud nodes, only three roots appeared at the end of the fourth day. The greatest number of roots appeared in the mixed B_1 and indolebutyric cultures, and root hairs were observed on one root 1.1 cm. in length. No growth of the buds occurred in the first 4 days.

In total root length, the indolepropionic cultures led all the series (except the mixed cultures) at both 12 and 16 days. At 16 days the second highest total

TABLE 1

TOTAL ROOT LENGTH, AVERAGE INDIVIDUAL ROOT LENGTH, AND TOTAL ROOT NUMBER OF NINE BUD NODES IN CULTURES AT 1 P.P.M. LENGTH VALUES IN CM.

Culture	Growth period											
	4 days			8 days			12 days			16 days		
	Root	Ave. root	Root no.	Root	Ave. root	Root no.	Root	Ave. root	Root no.	Root	Ave. root	Root no.
Indoleacetic	0.30	0.30	1	12.95	1.85	7	26.90	2.23	15	41.00	2.73	15
Indolepropionic	1.80	0.30	6	25.80	2.34	11	47.15	2.61	18	60.40	3.02	20
Indolebutyric	1.52	0.25	6	21.20	1.76	12	33.10	1.57	21	44.15	1.76	25
l-Tryptophane	2.57	0.51	5	20.60	2.94	7	33.45	3.34	10	33.95	3.39	11
Phenylacetic	0.30	0.30	1	7.75	1.29	6	19.15	2.38	8	31.75	2.88	11
Naphthaleneacetic	1.35	0.22	6	7.85	0.65	12	6.80	0.68	10	......	......	......
Vitamin B_1	0.00	0.00	0	5.90	1.96	3	15.90	2.65	11	25.67	2.56	11
Vitamin B_1 and indolebutyric	3.56	0.44	8	27.95	1.55	18	39.25	1.57	25	41.45	1.73	28
Average of two control series	0.25	0.16	1.5	7.50	1.77	5.5	16.75	2.64	8	29.30	2.90	9

root length occurred in the indolebutyric cultures. Although the indolepropionic cultures were the highest in total root length, they did not have the greatest average root length; they stood second to tryptophane, which had the highest average root length throughout the entire 16-day period.

TABLE 2

TOTAL SHOOT LENGTH OF NINE BUD NODES IN CULTURES AT 1 P.P.M. VALUES IN CM.

Culture	Growth period		
	8 days	12 days	16 days
Indoleacetic	3.90	6.90	9.10
Indolepropionic	3.80	6.25	7.55
Indolebutyric	2.70	2.65	4.10
l-Tryptophane	5.40	7.50	9.35
Phenylacetic	5.40	6.80	8.60
Naphthaleneacetic	5.50	6.20	....
Vitamin B_1	5.80	7.85	8.90
Vitamin B_1 and indolebutyric	2.95	2.25	3.70
Average of two control series	5.90	7.90	9.47

The greatest number of roots occurred in the mixed cultures throughout the 16 days. At 16 days this was three times the numbers formed in the controls. More primordia were stimulated to growth with the addition of B_1 to the indolebutyric solution, and particularly so at 8 and 12 days. Root elongation, and to some degree the number formed, were inhibited in the naphthaleneacetic cultures. The phenylacetic cultures showed no significant differences in root length or number over the controls.

Growth of the young shoots was definitely inhibited, both in the indolebutyric cultures and in the mixture of B_1 and indolebutyric. The indoleacetic exhibited no inhibition to shoot growth when compared with the controls. There appeared to be no stimulation of shoot growth in any of the other cultures of the series.

By 8 days root hairs were formed on roots in most of the indole-compound cultures. In one tryptophane culture they were sparsely formed on one root 4.6 cm. long. In naphthaleneacetic most of the roots had formed them, even on those as short as 3.0 mm. Likewise in indolepropionic most of the roots had

formed them. In indoleacetic one root only, 2.4 cm. in length, had sparse root hairs. In the mixed cultures most of the roots had developed root hairs. None were formed in the controls, in the B_1, or in the phenylacetic at this period or by the end of 16 days.

In one of the stem segments included in one indolebutyric culture a callus formed at the cut surface at the proximal end. Apparently in cutting the segment some meristematic tissue of the bud node remained, for at 4 days a callus had developed, and by 8 days a small bud and a root 2.4 cm. long had formed. By 12 days there were three roots, and the bud still was dormant. Further experiments with this type of cut segment failed to show any such regeneration.

EXPERIMENT 2.—This experiment compares the responses of bud nodes to indoleacetic acid and l-tryptophane with greater replication of cultures than in experiment 1. By changing the solutions, a more or less constant concentration was maintained. The conditions for this experiment were much the same as for experiment 1. The type of material, manner of cutting, and selection were the same. Light conditions (quality, intensity, and duration) were the same. The compounds used were indoleacetic acid and l-tryptophane in concentrations of 1 p.p.m. The single bud-node segments were grown in 200 ml. of solution as in the previous experiment. In experiment 2, six bud nodes were placed in each glass, and no stem segments were used. Measurements were made and the solutions changed every 4 days. Each compound, and also the controls in tap water, were tested in replicates of six. The temperature range was the same as in experiment 1. This experiment ran August 7–20, 1941.

The observations (table 3) follow closely those made in experiment 1. The stability of aqueous solutions of indoleacetic acid has often been questioned. Stock solutions of the acid usually turn brownish, even in storage at low temperatures. This color change has not been observed in the other indole acids, including tryptophane, under the same storage conditions. It is also the only one of the indole acids studied to develop a characteristic odor upon standing at room temperature.

TABLE 3

GROWTH OF BUD NODES IN CULTURES OF INDOLEACETIC AND L-TRYPTOPHANE AT 1 P.P.M. AND IN CONTROLS. LENGTH VALUES (CM.) REPRESENT TOTALS FOR THIRTY-SIX BUD NODES

Days	No. roots emerged	Total length of roots	Ave. root length	Total length of shoots
	l-Tryptophane			
4	13	4.35	0.61	10.52
8	17	41.95	2.46	20.40
12	21	56.15	2.67	37.33
	Indoleacetic acid			
4	18	7.71	0.83	8.39
8	24	37.35	1.55	13.92
12	38	62.10	1.63	30.76
	Tap water			
4	1	0.05	0.05	10.81
8	2	1.15	0.57	20.72
12	17	24.54	1.44	31.45

It was not until about 12 days that extensive rooting of the controls occurred. During the period of the experiment only the first root appeared, and no root hairs were formed on any of these roots. In contrast to the controls, rooting was relatively more extensive in the indoleacetic

and tryptophane series at all times. The indoleacetic cultures at all three observation periods exceeded the other series in total root number. For the first 8 days these were all first roots. By the twelfth day, six of the bud nodes in indoleacetic had produced second roots. In the tryptophane cultures it was not until the twelfth day that a second root appeared, and this occurred in only one bud node.

In total root length, the indoleacetic cultures led all the series throughout the 12-day period, as indolepropionic did in experiment 1. In that experiment indoleacetic was very near to the second highest (indolebutyric) only at 16 days. Again, although indoleacetic led, it did not have the greatest average root length. With the exception of the first 4 days, the tryptophane cultures (as in experiment 1) had the highest average root length of all the series.

Growth of the buds was definitely inhibited in the indoleacetic cultures. This was particularly evident in the first 8 days of growth. At the twelfth day the values for indoleacetic and the controls were not so greatly different. In tryptophane for the first 8 days, the shoot growth was no greater than the controls. At 12 days, however, a small increase in shoot growth over the controls occurred in tryptophane.

The first roots to produce root hairs grew in the indoleacetic cultures; they were visible on the fourth day. The two roots observed were 0.8 and 9.5 cm. in length. None were observed in the tryptophane at 4, 8, or 12 days. At 8 days no new root hairs had formed in any of the series. At 12 days one additional root, 1.3 cm. in length, was formed in one of the indoleacetic series. It was densely covered with root hairs.

Experiment 3.—The responses of bud nodes to a series of relatively high concentrations of indoleacetic acid were tested. The same type of bud node was used as in previous experiments. They were grown, however, in 2-liter crocks in the full light of the greenhouse. In this experiment ten such nodes were grown in 1 liter of tap water with added indoleacetic acid. Duplicate cultures were not grown. The usual measurements were made at 6 and 13 days (table 4). The concentrations were 1, 5, 10, 15, 20, 25, and 30 p.p.m. The solution was not changed throughout the experiment. The cultures were grown under average greenhouse conditions through February 10–23, 1942. The temperature of the solutions frequently measured 22° C.

At 6 days the maximum activity occurred in the range from 5 to 10 p.p.m. The maximum numbers of roots (ten and eleven, respectively) also emerged at these concentrations. This was true at 13 days as well. In contrast to the indolebutyric and indolepropionic in previous experiments, the second root emerged from very few of the nodes in the entire series, and no third or fourth roots had appeared. The higher light intensity may be somewhat inhibitory, and a possible deterioration of this substance in water solution may have resulted in the generally poor responses. In agreement with experiment 1, the responses to indoleacetic at 1 p.p.m. were not appreciable. At both 6 and 13 days the data for the buds were somewhat variable, but apparently inhibition was relatively greater in the range 25–30 p.p.m. There was little significant stimulation of shoot growth by any of the lower concentrations when compared with the control. No root-hair formation was observed on the regular bud-node roots.

Experiment 4.—The responses of bud nodes to a series of indolepropionic acid at high dilutions were tested. Cultures

at a concentration of 5×10^{-3} gm./l. (5 p.p.m.), and of a series from 1×10^{-3} (1 p.p.m.) to 1×10^{-10} gm./l., were made up in tap water. The same type of bud node and the same manner of cutting and selection were used as in previous experiments. The series was more extensive, however, consisting of four glasses containing four bud nodes each for each of the concentrations and for the controls in tap water alone. The data (table 5) are based on measurements from sixteen bud nodes for each concentration. The usual measurements were made at 4-day intervals. The solutions were changed twice after the beginning of the experiment. The cultures were grown under fluorescent light of an intensity of 200 f.c., February 4–17, 1942.

The limit of significant growth response was at 10^{-4} (table 5), apparent from the total bud-length values both at 8 and 12 days, and the root values at 12. The low light intensity was of value in showing the shoot response at low concentrations, but it was too low for much root growth at 10^{-3}, and no root growth occurred in the controls.

The greatest response in root and shoot growth occurred at 5 p.p.m. Root emergence was most abundant, and root hairs were formed. In one glass, a root 0.25 cm. long had formed a band of very short root hairs. Shoot growth was nearly equal to that in the 10^{-4} series.

While the values for shoot growth in both 10^{-3} and 5 p.p.m. were nearly the same, the character of the growth was

TABLE 4

SHOOT AND ROOT GROWTH IN CONCENTRATIONS OF INDOLEACETIC ACID. LENGTHS (CM.) ARE TOTALS FOR TEN BUD NODES

Days	Concentration (p.p.m.)							
	0	1	5	10	15	20	25	30
	Total shoot length							
6	3.39	3.43	3.89	3.94	2.93	3.52	2.65	3.20
13	7.45	5.98	7.96	5.84	4.47	8.87	3.31	5.75
	Total root length							
6	3.92	3.72	14.58	15.42	4.10	4.70	2.00	8.34
13	14.95	13.15	52.80	40.02	15.00	27.50	2.80	18.90
	Average root length							
6	1.30	1.57	1.45	1.92	2.05	0.78	2.00	1.66
13	2.13	1.47	5.28	3.63	5.00	4.58	2.80	4.72
	No. of roots							
6	3	4	10	8	2	6	1	5
13	7	9	10	11	3	6	1	4

entirely different. At 10^{-4} the buds actually opened, and the shoot grew in an apparently normal fashion both by an elongation of the existent secondary axis and by the growth of the apical meristem. In the 5 p.p.m. cultures the shoot growth was distinctly different. In many cases the sole increase in the length of the bud was in the excessive growth of the meristematic region at the base of the secondary axis. This may or may not be accompanied by elongation of the secondary axis proper or by growth of the apical meristem. Sporadic growth of the entire shoot occurred in two bud nodes. One grew to a length of 2.4 cm. and another to 1.7 cm. Most of the buds, however, were 4–5 mm. in length.

In the cultures at 10^{-4} the increased growth was more uniform. Six of the shoots were over 8 mm. in length, while most of the rest were 3.5–6 mm. In comparison with data from the other experiments, this was considerably less growth; but when compared with the rest of the dilutions from 10^{-5} to 10^{-10}, the response at 10^{-4} would appear to be significant. Apparently the presence of indolepropionic acid in the cultures was related to growth of both roots and shoots at the low intensity employed. Without this substance (as in the controls), or in dilutions greater than 10^{-4}, no root or shoot growth occurred.

EXPERIMENT 5.—This experiment concerned the responses of bud nodes to indolebutyric acid at low light intensities. Cultures were run in duplicate with four single whorled bud nodes in each glass. Daylight fluorescent tubes were suspended over a series of shelves constructed in stair-step fashion to provide the light intensities. Two cultures in tap water, and two in indolebutyric at 1 p.p.m., were placed on the shelves under approximately 20, 40, 100, and 200 f.c., with an additional series in continuous darkness. Observations were made at 3, 15, and 21 days (table 6). The experiment ran January 14–February 4, 1942.

At the end of 15 days a small amount of shoot growth had occurred in the controls at 200 f.c., but no roots had appeared. On the other hand, in the indole-

TABLE 5

TOTAL SHOOT AND ROOT LENGTHS AND TOTAL NUMBER OF ROOTS OF SIXTEEN NODES GROWN IN INDOLEPROPIONIC ACID AT LOW INTENSITY OF 200 F.C. LENGTHS IN CM.

CONCENTRATION (GM./L.)	GROWTH PERIOD								
	4 days			8 days			12 days		
	Total shoot	Total root	No. of roots	Total shoot	Total root	No. of roots	Total shoot	Total root	No. of roots
Control	4.81	......	......	4.47	......	......	4.70	......	......
1×10^{-10}	4.32	......	......	4.60	......	......	4.58	......	......
10^{-9}	4.54	......	......	4.67	......	......	4.81	......	......
10^{-8}	4.17	......	......	4.50	......	......	4.39	......	......
10^{-7}	4.34	......	......	4.89	......	......	4.45	......	......
10^{-6}	4.62	......	......	4.60	......	......	5.26	......	......
10^{-5}	4.58	......	......	4.67	......	......	4.76	......	......
10^{-4}	4.90	......	......	5.35	......	......	9.78	1.00	3
10^{-3}	4.67	......	......	4.75	1.39	2	5.51	1.04	2
5×10^{-3}	6.01	0.34	3	9.32	1.27	4	9.48	4.74	10

butyric cultures shoot growth was somewhat inhibited; while roots had emerged on over half the nodes. At 100 f.c. the controls showed no root emergence and little shoot growth, while in the indolebutyric, roots had emerged from over half the nodes and practically no shoot growth had occurred. At 40 f.c. there was no shoot or root growth in the controls, while in the indolebutyric small roots had emerged from half the nodes of shoot growth was still evident at all intensities and in the dark. Root growth occurred at all intensities, but not in the dark cultures. Only the first roots emerged by the end of 21 days in the indolebutyric cultures, and the maximum length of any root was 16 mm. at the highest intensity.

By the fifteenth day, the stumps of the nodes in the indolebutyric under the two lowest intensities, and in the dark as well, but there was no shoot growth. At the lowest intensity, 20 f.c., the controls showed no root or shoot growth, while the indolebutyric had a few roots emerging with no shoot growth. The cultures in the dark showed no growth of roots or shoots.

TABLE 6

TOTAL ROOT AND SHOOT LENGTHS OF BUD NODES UNDER VARYING LIGHT INTENSITIES WITH AND WITHOUT INDOLEBUTYRIC ACID (1 P.P.M.). VALUES (MM.) ARE TOTALS FROM EIGHT NODES

Light intensity	Growth period											
	3 days				15 days				21 days			
	Shoot		Root		Shoot		Root		Shoot		Root	
	o	Ibc	o	Ibc	o	Ibc	o	Ibc	o	Ibc	o	Ibc
Darkness.......	22.2	26.4	......	......	22.0	21.9	......	......	23.6	22.3	......	......
20 f.c..........	23.3	27.0	......	......	22.7	25.5	......	2.2	24.2	18.7	......	5.3
40 f.c..........	25.1	25.9	......	......	23.5	22.2	......	4.3	23.4	22.9	......	1.6
100 f.c..........	24.6	26.2	......	......	28.2	26.2	......	20.3	31.4	25.3	......	20.4
200 f.c..........	24.5	26.0	......	......	30.5	27.3	......	65.8	51.2	24.5	......	67.2

At the end of 21 days the responses were little changed from those at 15 days. No roots had emerged from the control cultures at any light intensity or in the dark. Shoot growth in the controls was entirely limited to the two higher intensities, 200 and 100 f.c., although very few nodes at the latter intensity showed much bud growth. In the indolebutyric series, inhibition (or no visible response) were beginning to decompose. Axillary buds and leaves, however, appeared intact and green in color. In contrast, the controls at all intensities and in the dark appeared to have a greater number of nodes with sound axis tissue.

In some preliminary experiments with 25-cm. shoots in tap water alone and in 1 p.p.m. indolebutyric grown under conditions similar to the present one, considerable stimulation of both root and shoot growth occurred in the indolebutyric at the lowest intensity employed (20 f.c.). But at the higher intensities (46, 130, and 200 f.c.) inhibition in root elongation occurred in indolebutyric, and greater elongation occurred in the

controls. At this concentration indolebutyric produced little if any inhibition in shoot growth at these higher intensities.

From experiments 4 and 5, and also from the work on histidine, it would appear that photosynthate was produced at these lower intensities, but that in tap water alone little could be utilized for root and shoot growth. It would seem that the addition of indolebutyric, indolepropionic, and possibly histidine, in some manner aided the utilization of the photosynthate, and possibly stimulated the synthesis of total solids (15). From the work of MITCHELL *et al.* (16, 17) and EYSTER (10), it would appear that these substances might be related to the enzymatic hydrolysis of the stored starch. Other workers (7) have also shown the interrelationships between auxins and cellular respiration, salt absorption, and cell enlargement. CLARK *et al.* (6) found that intermittent treatment with phenylacetic, phenylpropionic, and indoleacetic produced increased reproduction in sterile *Lemna* cultures, with 0.1 p.p.m. giving the greatest stimulation. Indoleacetic was the least effective, however. From the present experiments it would seem that some naturally formed growth substance or substances associated with normal metabolism is not formed in sufficient amount for growth at these lower intensities. With the addition of certain substances, however, growth may be promoted for a time.[1]

TABLE 7

TOTAL SHOOT LENGTHS OF THREE BUD NODES AT 16 DAYS

Substance	Concentration (p.p.m.)	Tube no. 1 (mm.)	Tube no. 2 (mm.)
Glycine	5	6.9	6.2
Tryptophane	5	7.3	7.0
Histidine	5	30.5	26.5
Histidine	25	8.2	7.7
Control	0	7.6	7.8

EXPERIMENT 6.—This experiment was concerned with histidine. For comparison of effects, two other amino acids, tryptophane and glycine, were used. Histidine was used in the form of the salt, l-histidine monohydrochloride. The solutions, all at 5 p.p.m. (with a duplicate series of histidine at 25 p.p.m.), were grown in large test tubes in 60 ml. of tap water. The same type of bud node was used as in previous experiments. The cultures were grown in duplicate, with three bud nodes in each tube, in the full light of the greenhouse under conditions described for experiment 3. This ran February 12–28, 1942.

This small-scale experiment seems to indicate that histidine in some manner is related to bud growth, while the other amino acids are not, at least under the conditions of this experiment (table 7). Growth in the tubes was slow, and it was not until at 8 days that the buds were noticeable, when five out of six buds had opened. At 16 days the buds in 5 p.p.m. histidine had grown appreciably, apparently confirming the work of SOLTYS *et al.* (23). With intact shoots of *Elodea* they noted a growth of the axillary buds in solutions of l-histidine monohydrochloride. In the above, 25 p.p.m. histidine was inhibitory to shoot growth. In the present experiment no roots appeared in the culture period of 16 days. This lack of rooting was chiefly related to the low light in-

[1] MEYER *et al.* (Ecology 24:393–399. 1943) have studied the apparent photosynthesis of certain submerged plants at various depths in Lake Erie. The compensation point for *Elodea canadensis* was attained at a depth of 8–10 m., with a light intensity range (1.03–0.27% of full sunlight) estimated from data of the present experiments to be equivalent to 99–27 f.c. of fluorescent light.

tensities prevailing during the growth period.

EXPERIMENT 7.—Cultures of l-histidine monohydrochloride at concentrations of 1, 2, 3, 4, 5, 8, 10, and 15 p.p.m. were grown at a light intensity of 350–450 f.c. Two series of cultures, one with short segments having one leaf whorl (lot 1) and one with longer segments having three whorls (lot 2), were grown at the concentrations named. The segments with one whorl had about eight leaves; those with three whorls had about sixteen. The data in table 8 are based on five segments for each culture. The experiment ran March 26–April 11, 1942.

TABLE 8

TOTAL ROOT AND SHOOT LENGTHS OF BUD NODES TREATED WITH HISTIDINE. LOT 1 HAD SHORT NODES WITH ONE WHORL OF LEAVES; LOT 2 HAD LONGER NODES WITH THREE WHORLS. VALUES (CM.) BASED ON SINGLE CULTURES OF FIVE NODES EACH

SOLUTION AND LOT	GROWTH PERIOD: 4 days		8 days		12 days		16 days	
	Shoot	Root	Shoot	Root	Shoot	Root	Shoot	Root
Control								
1	1.37	0.04	1.96	1.00	4.47	7.20	4.64	12.60
2	1.77	0.05	3.70	3.37	6.90	26.40	8.50	35.65
1 p.p.m.								
1	1.51	0.05	2.76	1.10	4.53	16.05	4.99	19.65
2	1.50	0.04	3.29	2.87	6.52	27.17	8.18	33.63
2 p.p.m.								
1	1.57	....	2.27	....	4.73	....	5.25	0.33
2	1.46	....	1.77	....	4.70	0.42	5.70	3.90
3 p.p.m.								
1	1.41	....	2.27	....	3.84	....	4.55	0.08
2	1.35	0.05	1.74	0.20	2.72	3.70	4.37	4.90
4 p.p.m.								
1	1.42	....	2.39	....	5.00	....	6.04	0.10
2	1.55	0.05	2.64	0.80	5.85	6.10	7.56	10.37
5 p.p.m.								
1	1.42	....	1.83	....	2.58	....	2.99	....
2	1.39	....	2.07	....	3.77	4.70	4.46	8.95
8 p.p.m.								
1	1.40	....	1.52	....	2.02	....	2.53	....
2	1.39	0.05	3.29	0.11	4.25	0.35	5.15	2.95
10 p.p.m.								
1	1.45	....	2.16	....	3.19	....	3.57	0.04
2	1.51	0.02	2.32	....	4.25	....	5.55	....
15 p.p.m.								
1	1.43	....	1.56	....	1.79	....	2.00	....
2	1.43	....	1.57	....	2.07	....	2.32	....

Table 8 shows the difference between the two lots with different-sized segments. With the larger ones (lot 2), root growth occurred in concentrations up to

8 p.p.m.; with the smaller segments (lot 1), little rooting occurred above 2 p.p.m. In the cultures of lot 1 there were practically no second roots formed, even at 1 p.p.m. In lot 2, second roots were formed only in the controls and in the 1 p.p.m. cultures. A few third roots were formed in the controls and in the 1 p.p.m. cultures of lot 2.

In lot 1, shoot inhibition was evident at 5 p.p.m., and also in lot 2 at the same concentration, with marked inhibition at 15 p.p.m. Inhibition of roots by concentrations higher than 1 p.p.m. may be in part an indirect one. All higher concentrations result in severe damage to the leaves of the segments. In the cultures having 1, 2, and sometimes 3 p.p.m., the leaves were bright green. At higher concentrations they became yellowish, and often the only green color was in the midveins. In older cultures these leaves became torn and decomposed. With leaf damage, sparse rooting generally followed.

EXPERIMENT 8.—This was designed to show possible correlations between light intensity and certain of the growth substances on the growth of excised bud nodes. In experiment 5 the cultures of indolebutyric at 1 p.p.m. maintained better growth of the segments at low intensities than the controls in tap water at such low intensities. In the present experiment, five bud nodes, each having one whorl of leaves, were grown in glass tumblers in 200 ml. of solution. The cultures were: duplicate cultures in tap water; duplicate cultures in histidine monohydrochloride at 5.0 p.p.m., and indolebutyric at 0.4 p.p.m.; and a single culture (five bud nodes) of histidine at 1.0 p.p.m. All solutions were made up with tap water.

The cultures were grown under different light conditions. One series of the cultures already described was placed in continuous darkness, another in daylight, and three were placed under three intensities of fluorescent light, obtained by the stair-step shelves as in experiment 5. A single fluorescent lighting unit, consisting of daylight tubes, was placed over the shelves. By this arrangement, three intensities of approximately 45, 200, and 400 f.c. were obtained. The cultures under fluorescent light were given 13 hours of light each day. The daylight series was grown under normal greenhouse conditions. The experiment ran April 22–May 8, 1942.

The cultures in the dark showed some root growth but practically no shoot growth (table 9). In many cases the bud scales opened, but no further growth occurred. The greatest number of roots (six at 16 days) and the greatest total root length (2.23 cm.) occurred in the dark series of histidine cultures (5 p.p.m.). While a number of roots emerged in the indolebutyric cultures, the roots remained very short.

In the cultures at the low light intensity of 45 f.c. there was marked difference in shoot growth. Both histidine at 5 p.p.m. and indolebutyric at 0.4 p.p.m. had a greater total shoot length than the controls at all three periods—8, 12, and 16 days. This effect of histidine is in agreement with a previous test. At low intensities, histidine and indolebutyric acid would appear to be associated with increased shoot growth. While the greatest number of roots (five) occurred in the indolebutyric at 16 days, there was little growth in length of these roots at this low intensity. Considerably greater total root length occurred in the 1 p.p.m. histidine than in the control.

At the higher intensities, 200 and 400 f.c., there was greater growth of both roots and shoots in most of the cultures.

TABLE 9

GROWTH RESPONSES OF CULTURES UNDER DIFFERENT LIGHT INTENSITIES. SHOOT AND ROOT LENGTHS (CM.) ARE TOTAL LENGTHS FROM TEN NODES (EXCEPT AS NOTED) IN EACH SERIES. NUMBER OF ROOTS REPRESENTS TOTALS FOR TEN NODES (EXCEPT AS NOTED)

Culture	Concentration (p.p.m.)	Growth period: 8 days			12 days			16 days		
		Shoot	Root	No. of roots	Shoot	Root	No. of roots	Shoot	Root	No. of roots
		Darkness								
Control	0	3.20	0.25	2	3.39	0.46	3	3.21	1.24	3
Indolebutyric	0.4	3.02	0.35	4	3.39	0.44	4	3.34	0.70	5
Histidine	5.0	2.92	0.69	4	3.01	1.28	4	3.22	2.23	6
Control*	0	1.62	0.15	1	1.65	0.14	1	1.66	0.82	2
Histidine*	1.0	1.56	0	0	1.69	0	0	1.77	0	0
		45 foot candles								
Control	0	3.23	0.45	2	3.68	2.58	3	4.10	2.75	2
Indolebutyric	0.4	4.57	0.25	3	5.47	0.49	4	6.43	0.10	5
Histidine	5.0	5.27	0.45	2	6.03	2.40	2	6.50	4.24	3
Control*	0	1.53	0.05	1	1.72	0.08	1	1.99	0.05	1
Histidine*	1.0	1.94	1.05	2	2.21	4.95	2	2.44	5.75	2
		200 foot candles								
Control	0	7.54	3.97	4	9.60	5.15	3	11.40	3.85	4
Indolebutyric	0.4	6.41	6.37	4	8.57	15.08	6	10.01	19.25	6
Histidine	5.0	5.91	3.07	2	7.84	6.05	3	9.22	6.79	4
Control*	0	3.74	0.17	2	5.27	1.35	1	6.00	1.60	2
Histidine*	1.0	2.87	0.65	1	3.62	4.69	2	3.64	7.80	2
		400 foot candles								
Control	0	8.74	4.08	7	11.45	13.65	8	13.09	21.02	7
Indolebutyric	0.4	7.80	9.72	4	8.90	12.20	6	8.80	13.05	7
Histidine	5.0	6.92	0.25	1	8.83	1.89	2	9.78	2.78	3
Control*	0	4.87	0.42	4	6.40	7.05	4	7.04	8.32	3
Histidine*	1.0	4.37	1.51	5	5.65	12.75	5	6.10	16.00	4
		Daylight								
Control	0	6.38	0.30	3	8.92	0.80	5	11.32	2.02	7
Indolebutyric	0.4	5.58	5.30	4	7.87	1.89	8	10.33	8.20	10
Histidine	5.0	5.14	0	0	6.82	0.15	3	8.69	0.10	2
Control*	0	3.20	0	0	4.68	0.10	1	5.90	1.50	4
Histidine*	1.0	2.92	0.27	3	4.30	0.33	2	5.31	1.11	4

* Values for single control culture (five nodes only) and for single histidine culture at 1 p.p.m., respectively.

In the controls shoot growth was higher at these two intensities than at the lower ones and in daylight. Root growth in the controls reached a maximum at 16 days at 400 f.c. This was much greater than the value in the daylight controls. The total root growth in the indolebutyric was the highest at 16 days at 200 f.c. In the histidine at 5 p.p.m., inhibition of shoot growth occurred at both 200 and 400 f.c., while root growth was somewhat higher than the controls at 200 f.c. and decidedly inhibited at 400 f.c. In the 1 p.p.m. cultures of histidine there appeared to be greater root growth than in the controls at both 200 and 400 f.c.

In the daylight series, the total root and shoot lengths in almost every culture were considerably lower than those values at 400 f.c. While there may have been several other factors, such as greater temperature fluctuation, the very high light intensity was probably the chief.

It is interesting to note the differences in responses of this experiment and experiment 5. In this experiment some roots appeared on nodes in the control cultures in the dark and in the controls under the low intensities; in experiment 5 no roots appeared in the controls in the dark or under any of the low intensities. Possibly the time of year at which these nodes were cut was largely responsible for these differences. The nodes in experiment 5 were cut on January 14; those in experiment 8 were cut on April 22. Owing to cloudy weather, and the low temperature maintained in the house, there was little growth in the culture tanks during the winter months. Bausor (1) has pointed out that variations in the rooting of cuttings at different times of the year may be related to the reserve carbohydrate.

Experiment 9.—This was concerned with the responses of bud nodes to compounds at relatively high concentrations (5 p.p.m.). These bud nodes were cut differently. Instead of the one whorl of leaves on the bud segment, three successive whorls were left. The segments were longer, about 2.5–3.0 cm., thus having greater leaf and stem area than those previously used. A higher fluorescent light intensity, 420–440 f.c., was employed. The segments were grown five in each glass tumbler containing 200 ml. of solution. The water was obtained by filtering that from the tanks in which the stock cultures were growing. The temperatures during the daytime were generally 24°–28° C. The experiment ran February 19–28, 1942.

At higher light intensities, and with larger segments, growth of the roots and buds was more rapid. This greater growth may be related to the photosynthate (1). Table 10 shows the well-defined responses to certain of the compounds. The responses to treatment with the indole compounds were much the same as reported earlier. At 8 days indolepropionic acid was the highest of all in bud stimulation. Again this was due to a combination of the stimulation of the basal meristem of the secondary axis and some general growth of the latter. At 8 days the indolebutyric culture had the greatest percentage of roots per segment. Both the first and second roots had elongated in all five segments, while a third root emerged in one. Indoleacetic chiefly stimulated growth of the first root; in only two segments had the second root emerged. The average root length was great here also. The average was quite low in indolepropionic and indolebutyric but high again in tryptophane. The greater root elongation occurring in the tryptophane may be related to its role as an amino acid. This is apparent when glycine (which has the amino group but

no indole configuration) produces about the same responses. Growth in histidine was not pronounced, but shoot growth was somewhat greater than the controls.

Phenylacetic and thiophenyl acetamide did not produce definite gross responses in any of the cultures. Naphthaleneacetic is definitely inhibitory and approaches toxicity at 5 p.p.m. The three vitamins appear to stimulate rooting to a limited degree, especially ascorbic acid.

EXPERIMENT 10.—This was designed to study the responses of excised segments to a series of amino acids and some other growth substances. The segments of three whorls were grown under conditions similar to those of preceding experiments, under fluorescent lights at an average intensity of 400 f.c. Duplicate cultures of five segments each were grown for a period of 20 days. The substances used were histidine monohydrochloride, l-tryptophane, glycine, urea, asparagine, thiamin hydrochloride, nicotinic acid, ascorbic acid, and calcium pantothenate. The solutions were made up with tap water at a concentration of 5 p.p.m., and the original solutions were replaced with fresh ones at 17 days. The experiment ran March 31–April 20, 1942.

Ascorbic acid and calcium pantothenate were associated with the highest rooting activity at 12, 16, and 20 days. Plants in these, together with B_1 and tryptophane, cultures were consistently high in rooting activity during the 20 days. Responses in cultures of glycine, nicotinic acid, and asparagine, while not so marked as in the cultures previously listed, were still higher in root growth than the controls. Histidine was almost completely inhibitory to root growth (table 11). Although ascorbic acid (vitamin C) has been reported to oxidize readily, this substance or its changed form appeared to stimulate root growth.

In the number of roots formed by the end of the 20-day period, pantothenate and tryptophane led (twenty and twenty-one, respectively), while the controls had a total of ten roots. The lowest numbers were in histidine and asparagine—three and seven, respectively. The cultures containing ascorbic acid, B_1, and urea formed more roots than the controls. Not more than one primordium per node was stimulated to growth in the histidine cultures, and then only a few roots were formed. At the end of 20 days, second roots only were formed in the B_1, glycine, asparagine, and control series. The largest number (three) of third roots was in the tryptophane cultures, while two segments had third roots in each of the cultures of ascorbic, nicotinic, pantothenate, and urea.

The growth differences of the shoots were not so striking. Definite inhibition of the shoot occurred with histidine and to a lesser extent with tryptophane. No very great increase of shoot growth could

TABLE 10

TOTAL SHOOT AND ROOT LENGTHS AND TOTAL NUMBER OF ROOTS OF BUD NODES GROWN AT 5 P.P.M. LENGTH VALUES (CM.) ARE TOTAL LENGTHS OF SHOOTS AND ROOTS OF FIVE BUD NODES

Culture	Growth period					
	4 days			8 days		
	Total shoot	Total root	No. of roots	Total shoot	Total root	No. of roots
Indoleacetic	1.55	0.58	4	3.80	18.80	7
Indolepropionic	2.15	0.68	4	6.05	4.75	8
Indolebutyric	1.98	0.73	4	3.72	9.16	11
l-Tryptophane	1.67	0.78	5	4.80	20.65	7
Glycine	2.17	0.64	5	4.50	21.26	7
Histidine	2.00	0.43	5	4.95	12.47	5
Phenylacetic	1.46	0.10	2	4.23	7.15	4
Thiophenyl acetamide	1.47	0.26	3	3.45	3.83	4
Naphthaleneacetic	1.82	0.30	2	2.77	0.30	2
Nicotinic acid	1.52	0.09	2	3.63	12.05	4
Ascorbic acid	1.85	0.15	1	5.15	12.75	7
Vitamin B_1	1.71	0.25	5	4.25	12.70	5
Control	1.65	0.40	4	3.58	9.25	6

be correlated with any of the compounds at 5 p.p.m.

Inhibitory responses of shoot growth in tryptophane are in contrast to the general shoot stimulation noted in indolepropionic cultures in other experiments. The total shoot length of the tryptophane cultures (10.99 cm.) at 20 days was considerably lower than the controls (16.86 cm.). In other experiments, shoots in indolepropionic have generally been much greater than the controls. This differential response would not seem to be correlated with root growth, since root stimulation is much the same in cultures of these two compounds. This difference of response in shoot elongation was also evident in experiment 13.

In experiments with a greater range of concentrations of glycine—not reported here—the greatest total root lengths and the greatest number developed in 1 p.p.m. Shoot growth could not be correlated very well with the various concentrations. No inhibition in shoot growth occurred, however, even at the high concentration of 100 p.p.m. This increased growth in glycine, and in urea also, may be due in part to utilization of the amino nitrogen present in these compounds. WHITE (25) has found that glycine alone could substitute for all the amino acids formerly required in the nutrient used for certain root-tip cultures.

TABLE 11

TOTAL ROOT AND SHOOT LENGTHS OF NODES GROWN IN CULTURES OF DIFFERENT COMPOUNDS AT 5 P.P.M. VALUES (CM.) ARE TOTALS FROM TEN NODES

SOLUTION	GROWTH PERIOD									
	4 days		8 days		12 days		16 days		20 days	
	Shoot	Root	Shoot	Root	Shoot	Root	Shoot	Root	Shoot	Root
Urea	2.78	....	6.57	1.32	11.83	16.33	15.25	52.92	16.70	59.55
Vitamin B_1	3.01	0.29	6.58	6.16	11.40	25.97	15.45	49.88	17.15	72.50
Histidine	3.00	0.03	4.02	0.17	5.60	0.35	7.84	2.20	9.35	3.18
Ascorbic	2.99	0.18	6.52	9.75	11.03	40.69	15.06	63.62	17.55	76.39
Nicotinic	2.75	0.05	3.79	1.19	6.89	7.88	6.55	13.50	14.34	29.33
Asparagine	2.74	....	4.71	0.70	8.17	6.22	12.75	17.02	13.79	21.45
Glycine	3.08	0.10	4.93	0.44	9.00	6.65	12.85	23.21	16.15	33.28
Calcium pantothenate	2.99	0.09	6.16	3.88	10.61	36.29	14.35	62.40	15.76	73.20
Tryptophane	2.93	0.86	3.67	11.14	4.36	31.50	6.53	52.67	10.99	62.53
Control	3.03	....	4.91	....	9.00	....	14.07	9.84	16.86	16.90

EXPERIMENT 11.—A series of cultures with concentrations of alpha naphthalene acetamide at 0.5, 2.5, 5.0, 7.5, and 10.0 p.p.m. were grown under fluorescent lights at an average intensity of 400 f.c. Duplicate cultures, each with five bud nodes of three leaf whorls, were used. The experiment ran April 2–22, 1942.

Roots appeared rapidly in the 5 p.p.m. cultures, for at 3 days roots had emerged from nine of the ten nodes; while only two nodes in the controls had rooted (table 12). At 12, 15, and 20 days the greatest total root length, and the greatest number of roots, occurred at 2.5 p.p.m. At 15 and 20 days both the root lengths and the number emerging were inhibited at 10 p.p.m. When the five longest roots in each culture were aver-

aged, the greatest average root length was 6.7 cm. for the 12- and 15-day periods at 2.5 p.p.m. (6.2 cm. at 12 days in 0.5 p.p.m. cultures), while the controls averaged only 3.9 cm. at 12 days and 4.0 cm. at 15 days. At 20 days the averages were much as just described.

In other experiments with lower concentrations of naphthalene acetamide, not reported here, the roots attained great lengths. In the controls the roots began to die when reaching lengths of 4 or 5 cm., while in cultures at 0.2, 0.3, 0.4, and 0.5 p.p.m. roots attained lengths of 8, 9, and 10 cm. The longest root ever measured in cultures grown in glass tumblers was 11.8 cm. at 17 days in the 0.2 p.p.m. In these experiments root hairs were observed on roots at 3.0 p.p.m.

In the present experiment fourth roots appeared at 15 days in the 0.5, 2.5, and 5 p.p.m. cultures. At this period the 2.5 p.p.m. cultures were the only ones in which all nodes had third and fourth roots. No third or fourth roots had yet appeared in the controls, eight nodes having rooted, with only three having the second root. At 20 days fifth roots appeared in the 0.5, 2.5, and 5 p.p.m. cultures; no fourth or fifth roots had appeared in the controls or at 10 p.p.m.

Root hairs were observed on some of the distal portions of the longer roots in the 0.5 p.p.m. at 15 days. Root hairs were not observed in the higher concentrations. There was little correlation of shoot growth with the various concentrations. At 10 p.p.m., however, shoot growth appeared to be definitely inhibited.

There are striking differences in the responses to naphthaleneacetic acid and to naphthalene acetamide at the same concentrations. Root-hair formation is very profuse with naphthaleneacetic at 1.0 p.p.m., and this concentration is highly inhibitory to root elongation, since most roots rarely attain lengths greater than 1–2 cm. This concentration is also inhibitory to shoot growth. In naphthalene acetamide, root-hair formation is sparse and has been observed at concentrations of 0.5 and 3.0 p.p.m., while inhibition in root elongation is not very marked, even at concentrations of 10 p.p.m.

TABLE 12

TOTAL SHOOT AND ROOT LENGTHS AND TOTAL NUMBER OF ROOTS OF TEN NODES IN ALPHA NAPHTHALENE ACETAMIDE. LENGTHS (CM.) ARE TOTALS FOR TEN NODES

Concentration (p.p.m.)	Growth period: 3 days			8 days			12 days			15 days			20 days		
	Shoot	Root	No. of roots	Shoot	Root	No. of roots	Shoot	Root	No. of roots	Shoot	Root	No. of roots	Shoot	Root	No. of roots
0........	3.25	0.15	2	6.52	1.85	4	11.70	11.54	7	16.99	29.59	11	20.65	39.05	12
0.5......	3.17	0.38	5	7.41	12.35	9	12.36	51.32	10	16.75	88.97	27	17.60	80.95	31
2.5......	3.32	0.90	6	5.12	25.43	13	7.04	72.56	23	8.75	103.00	33	9.02	106.66	40
5.0......	3.50	1.35	9	4.94	33.28	13	7.49	48.92	19	11.91	69.10	31	11.05	73.18	33
7.5......	3.56	0.69	6	4.32	12.49	10	5.67	31.98	13	7.05	42.04	21	7.90	43.50	26
10.0.....	2.95	0.66	7	3.68	13.22	10	4.01	20.02	12	4.64	22.75	13	4.67	18.45	12

EXPERIMENT 12.—In experiment 1 it was observed that, in a mixture of vita-

min B_1 and indolebutyric acid (each at 1 p.p.m.), root emergence was somewhat greater than in the single cultures of indolebutyric. This mixture was tried again, using each at 5 p.p.m. Four three-whorled segments were used in each glass, with each series containing four glasses. Filtered water from the culture tank was used. The cultures were grown under daylight fluorescent lights of about 400 f.c. The experiment ran March 13–April 1, 1942.

At 12 days there was marked inhibition of shoot growth in the indolebutyric cultures, while the difference between the mixture and the control was not so great (table 13). There was about a 25% greater total root length in the B_1 cultures at 12 days than in the controls. The indolebutyric and the mixture were about the same—only about one-third as great as the controls in total root lengths. A comparison of the five greatest root lengths in each of the series showed the longest in the B_1 cultures. At 16 days there appeared to be a little less inhibition of shoot growth in the mixture than in the single indolebutyric cultures.

At 12 days there were about four times as many nodes having third roots in the mixture as in the controls. At 16 days fourth roots were formed in the indolebutyric and in the mixture, while in the controls and in the B_1 cultures the maximum number per node was three. A fifth root was produced on one node in the mixture at this growth period. Except for a greater stimulation of third and fourth roots, the greater total number of primordia stimulated in the mixture at 5 p.p.m. was less evident than in the mixture at 1 p.p.m. in experiment 1.

EXPERIMENT 13.—This was designed to study the elongation rates of apical segments in various substances in the light and in the dark. Apical segments 4 cm. in length were cut from shoots of *Elodea* growing in the culture tanks. Four segments were placed in each glass tumbler, and two series, one in light and one in continuous darkness, were run simultaneously. There were no duplicate cultures in either series. The segments were grown in a nutrient solution consisting of 0.0045 mol. $MgSO_4$, 0.0045 mol. KH_2PO_4, 0.0060 mol. $Ca(NO_3)_2$, together with the minor elements diluted 1:20. The various substances were then added at a concentration of 5 p.p.m. Those used were indoleacetic, indolepropionic, indolebutyric, naphthaleneacetic, alpha naphthalene acetamide, l-tryptophane, vitamin B_1, ascorbic acid, calcium pantothenate, and l-histidine monohydrochloride. The cultures in the light were grown under fluorescent light for 13 hours each day at about 400 f.c. The other series was grown in continuous darkness, except for the very short time used in measuring. Measurements of the segments in the dark were made with the aid of a faint green light approximately every 20 hours. The temperatures of the two series did not differ greatly. For example, on May 6 at 7:30 P.M. the water temperature was 23.8° C. in the dark series and 22.0° C. in the light series. The experiment ran May 7–13, 1942.

Growth rates were calculated on the basis of the total growth increment of the four segments in each culture for each of the approximately 20-hour periods (table 14). The control culture referred to is the one at the 1:20 dilution, since for comparison another control in 1:1 nutrient was also used. Preliminary experiments with apical segments had shown that if the leaves were stripped back about 10–15 mm. from the growing point, there was some elongation in tryptophane at 5 p.p.m. in the dark. No expansion of the tiny leaves or growth at

the apical meristem appeared to take place. Increase in length of the segments was apparently due entirely to elongation of the internodes, principally those in the apical third of the segment.

The segments elongated somewhat during the first 21 hours in all cultures of both series. The highest growth rate for this first 21 hours, 0.30 cm./hr., was in the indoleacetic dark culture, while the rate was 0.13 cm./hr. in the dark control. The second highest rate for this period, 0.29 cm./hr., was in the naphthaleneacetic dark culture. The greatest decrease in growth rate in the second period (41 hours) was in both the dark and light series in the indoleacetic cultures. This possibly indicates a deterioration of the compound in water solution, since the cultures of the other indole compounds generally showed a higher growth rate at the end of the 41-hour period.

The naphthalene acetamide dark culture was the only one in the entire series which had a higher growth rate at the end of 41 hours than at the end of 21 hours. The highest growth rate maintained in the later periods was in the naphthaleneacetic dark culture, with 0.14 cm./hr. at the end of 102 hours. The rate in this culture at 129 hours was also the highest for any of the cultures.

Practically no stimulation of the segments occurred in the tryptophane cultures, either in the dark or the light series. In fact, tryptophane appeared to inhibit elongation in the light series. The ascorbic-acid, pantothenate, and histidine cultures all had greater growth rates than the controls in the dark; while in the light there was no appreciable stimulation. The growth rate in the histidine dark culture remained relatively high during the first three growth periods (up

TABLE 13

TOTAL SHOOT AND ROOT LENGTHS AND TOTAL NUMBER OF ROOTS OF BUD NODES GROWN IN 5 P.P.M. OF INDOLEBUTYRIC ACID AND 5 P.P.M. OF VITAMIN B_1, AND IN MIXTURE OF THESE TWO SUBSTANCES EACH AT 5 P.P.M. LENGTH VALUES (CM.) ARE TOTAL LENGTHS OF SHOOTS AND ROOTS OF EIGHT NODES. VALUES FOR TWO LOTS (EACH OF TWO CULTURES) INCLUDED

Culture and lot	Growth period											
	4 days			8 days			12 days			16 days		
	Shoot	Root	No. of roots	Shoot	Root	No. of roots	Shoot	Root	No. of roots	Shoot	Root	No. of roots
Control												
1.	2.81	0.33	5	6.25	9.40	7	10.93	34.64	16	15.65	81.05	22
2.	2.95	0.46	6	6.21	12.80	9	11.10	38.88	16	15.85	86.15	22
Vitamin B_1												
1.	3.01	0.47	5	7.40	15.44	7	12.35	49.97	17	17.15	112.45	22
2.	2.70	0.40	4	6.55	13.25	8	10.55	50.33	14	13.95	93.09	21
Indolebutyric												
1.	3.08	1.05	8	4.04	6.64	13	6.91	13.90	17	10.60	21.60	22
2.	2.76	0.92	5	3.88	4.30	11	6.35	13.15	17	11.05	22.50	22
Mixture												
1.	2.83	0.85	7	5.73	6.32	13	8.55	13.75	20	12.90	18.80	22
2.	3.15	1.31	7	4.96	5.40	12	10.80	12.82	20	18.20	23.05	26

TABLE 14

TOTAL LENGTHS, GROWTH INCREMENT, AND MEAN HOURLY GROWTH RATE IN CM./HR., FOR TOTAL OF FOUR SEGMENTS IN EACH CULTURE OF LIGHT AND DARK SERIES FOR EACH GROWTH PERIOD. MEASUREMENTS (CM.) ARE TOTAL VALUES FOR THE FOUR SEGMENTS IN EACH CULTURE

SOLUTION		HOURS											
		21		41		62		80		102		129	
		Light	Dark	Light	Dark	Light	Dark	Light	Dark	Light	Dark	Light	Dark
Indoleacetic	T*	19.80	22.22	21.72	26.35	22.75	26.90	23.23	27.39	23.85	27.40	24.87	27.95
	I	3.80	6.22	1.92	4.13	1.03	0.55	0.48	0.49	0.62	0.01	1.02	0.55
	R	0.18	0.30	0.10	0.21	0.05	0.03	0.03	0.03	0.03	0.00	0.04	0.02
Indolepropionic	T	19.29	21.98	20.50	27.60	22.20	32.45	23.30	35.60	24.80	37.50	26.29	38.98
	I	3.29	5.98	1.21	5.62	1.70	4.85	1.10	3.15	1.50	1.90	1.49	1.48
	R	0.16	0.28	0.06	0.28	0.08	0.23	0.05	0.15	0.07	0.09	0.06	0.05
Indole-butyric	T	18.96	21.20	19.91	25.21	20.40	29.61	21.15	32.07	22.20	33.90	23.10	35.18
	I	2.96	5.20	0.95	4.01	0.53	4.40	0.71	2.46	1.05	1.83	0.90	1.28
	R	0.14	0.25	0.05	0.20	0.03	0.21	0.04	0.14	0.05	0.08	0.03	0.05
Naphthaleneacetic	T	19.27	22.03	21.67	27.34	24.82	32.05	26.95	35.32	28.35	38.40	29.55	40.00
	I	3.27	6.03	2.40	5.31	3.15	4.71	2.13	3.27	1.40	3.08	1.20	1.60
	R	0.16	0.29	0.12	0.27	0.15	0.22	0.12	0.18	0.06	0.14	0.04	0.07
Naphthalene acetamide	T	18.30	21.75	20.10	27.65	21.45	31.40	22.38	33.05	23.65	34.30	26.00	35.40
	I	2.30	5.75	1.86	5.90	1.29	3.75	0.93	1.65	1.27	1.25	2.35	1.10
	R	0.11	0.27	0.09	0.30	0.06	0.18	0.05	0.09	0.06	0.06	0.09	0.04
Vitamin B_1	T	16.57	19.18	16.75	21.35	17.11	23.07	17.23	24.00	17.72	24.70	18.13	24.21
	I	0.57	3.18	0.18	2.17	0.36	1.72	0.12	0.93	0.49	0.70	0.41	0.00
	R	0.03	0.16	0.01	0.11	0.02	0.08	0.01	0.05	0.02	0.03	0.02	0.00
Ascorbic	T	16.60	20.03	16.74	23.27	17.27	25.05	17.30	27.60	17.97	26.45	18.58	26.92
	I	0.60	4.03	0.10	3.24	0.53	1.78	0.03	2.55	0.67	0.00	0.61	0.00
	R	0.03	0.19	0.04	0.16	0.03	0.08	0.00	0.14	0.03	0.00	0.02	0.00
l-Histidine	T	16.80	19.43	17.00	22.19	16.98	22.55	17.20	23.08	17.74	23.80	18.34	24.12
	I	0.80	3.43	0.20	2.76	0.00	0.36	0.22	0.53	0.54	0.72	0.60	0.32
	R	0.38	0.16	0.01	0.14	0.00	0.17	0.01	0.03	0.02	0.03	0.02	0.01
l-Tryptophane	T	17.31	19.60	17.77	22.55	18.07	23.70	18.11	24.75	18.58	25.45	19.19	25.75
	I	1.31	3.60	0.46	2.95	0.30	1.15	0.04	1.05	0.47	0.70	0.61	0.30
	R	0.06	0.02	0.02	0.15	0.01	0.05	0.00	0.05	0.02	0.03	0.02	0.01
Calcium pantothenate	T	16.99	18.25	17.32	19.24	17.88	19.40	18.11	19.50	18.67	19.75	19.45	19.95
	I	0.99	2.25	0.33	0.99	0.56	0.16	0.23	0.10	0.56	0.25	0.78	0.20
	R	0.05	0.11	0.02	0.05	0.03	0.01	0.01	0.01	0.03	0.01	0.03	0.01
Nutrient (1:1)	T	16.57	17.48	17.05	19.70	17.76	20.70	18.27	21.34	18.83	22.05	19.81	22.90
	I	0.57	1.48	0.48	2.22	0.71	1.00	0.51	0.64	0.56	0.71	0.98	0.85
	R	0.03	0.07	0.02	0.11	0.03	0.05	0.03	0.04	0.03	0.03	0.04	0.03
Nutrient (1:20)	T	17.28	18.70	17.72	20.15	17.24	20.75	18.75	20.72	19.38	22.30	22.72	21.17
	I	1.28	2.70	0.44	1.45	0.00	0.60	1.51	0.00	0.63	1.58	3.34	0.00
	R	0.06	0.13	0.02	0.07	0.00	0.03	0.08	0.00	0.03	0.07	0.12	0.00

* T, total length of four segments in each culture; I, total growth increment of the four segments; R, mean hourly growth rate in cm./hr. for total growth increment of the four segments for each period.

to 62 hours); while inhibition definitely occurred in the light culture throughout the entire 129 hours.

In all but one of the light cultures the growth rate was lower up to the end of 41 hours than the rate in the dark series. The highest growth rate in the light series occurred in the first 21 hours in the indoleacetic, 0.18 cm./hr.; in the control it was 0.06 cm./hr. There was prolonged growth rate in the naphthaleneacetic light culture. This rate at the end of 62 hours, 0.15 cm./hr., was the highest of all in the light series at that period. The least inhibition of the growth rate by light in any of the cultures was in the indoleacetic culture in the first 21 hours. A clue to the relationships of auxins and light is to be found in the work of EYSTER (10).

Definite inhibition in growth rate occurred in the ascorbic, B_1, and histidine light cultures: all were lower than the control. Calcium pantothenate and tryptophane were inhibitory to growth for all periods after the first 21 hours.

In the matter of the greatest elongation in the dark, one segment in the indolepropionic solution measured 10.9 cm., the greatest in the entire series. This was a 36% increase over the original 4-cm. segment in the growth period of 129 hours. The greatest total increment for the four segments in any one culture for the total growth period of 129 hours was in both the dark and light naphthaleneacetic cultures. The next highest in total increment were the dark and light cultures in indolepropionic.

The presence of the amino group in the l-tryptophane molecule appears radically to alter the properties of this substance when the responses of indolepropionic are considered. In the previous experiments with indolepropionic, root elongation was somewhat inhibited, but at the same time elongation of the secondary axis was greatly increased. Tryptophane, however, does not stimulate axis elongation. The theory that growth responses resulting from the application of tryptophane are due to indoleacetic acid, the latter originating through chemical change of tryptophane, has been questioned by KRAUS (13). The experiments with apical segments indicated that tryptophane did not show characteristic indoleacetic responses. It must be recognized, however, that tryptophane, as well as the other substances used in these experiments, may undergo a change in non-sterile aqueous solutions.

ROOT-HAIR FORMATION

The rooting reactions of species of this genus have been noted (22, 26, 8). Ordinarily, the free-growing roots of *Elodea* do not form root hairs in water. Generally such hairs are produced (*a*) in darkness; (*b*) upon penetration of the root into soil; (*c*) by treatment with ethylene gas; and (*d*) when the roots are in contact with a medium such as quartz sand.

In some preliminary investigations upon the effects of indolebutyric acid on 25-cm. shoots of *Elodea*, it was noted that root hairs were formed on most of the roots in concentrations of 1 p.p.m. This occurred in cultures grown in the full light of the greenhouse as well as in those grown under fluorescent lights of 200 f.c. With the use of excised bud nodes in the experiments described here, it has been observed that—in addition to indolebutyric acid—indolepropionic acid, naphthaleneacetic acid, alpha naphthalene acetamide (and to a lesser degree indoleacetic acid and l-tryptophane) stimulated root-hair formation. Of all the different types of compounds used in these experiments, only those

with the indole or naphthalene ring system—and acid side chains of two up to four carbons—were associated with root-hair formation.

CORMACK (8) states that certain short cells are cut off early in the development of the epidermis. These have been termed piliferous cells, since these are the only ones which produce hairs. In the case of treatment with indolebutyric acid at least, these special cells are the only ones to develop root hairs. On roots growing in water, the piliferous cells soon lose their identity and in the mature region of the root have the same shape as the rest of the elongated epidermal cells. In roots penetrating soil, the piliferous cells develop hairs and then these cells elongate as do the rest. When grown in 5 p.p.m. indolebutyric acid, the roots were rather rigid. Root hairs were formed abundantly, and elongation was inhibited to some degree. Piliferous cells, all with root hairs, still retained their characteristic cell shape, even in the mature region of the root. There was considerable thickening of the walls of the cells in the outer regions of the cortex. In some, the smaller intercellular spaces had been almost obliterated by the extensive thickening of the bounding walls. The stelar region seemed not to have undergone any thickening.

In root hairs formed in 1 p.p.m. indolepropionic acid, cells were often forked terminally. This was more evident when developed in 5 p.p.m. In the latter case the hair cells were not only frequently forked, but often many buds and branches appeared. Often the cells were club-shaped, with the terminal portion considerably swollen.

CORMACK (8) found that ethylene gas stimulated root-hair formation in *Elodea*. Although he attributed this to an inhibition in cuticle formation, BORGSTRÖM (2)—on the basis of his own work and data from the work of CROCKER, HITCHCOCK, and ZIMMERMAN (9) and others—has proposed that root-hair formation is an auxin response. He states that ethylene produces a transverse movement of auxin from the phloem to the epidermis.

In the light, root hairs do not develop on free-growing roots, even under the low intensities used in these experiments. Large segments placed in continuous darkness in tap water did form roots with hairs. This inhibition of root-hair formation in the light is probably related to auxin utilization. The somewhat inhibitory action of light on the elongation of apical segments is evident in experiment 13. EYSTER (10) has suggested that light accelerates the rapidity with which enzymes are bound to a colloidal carrier, consequently making them less free to act, and that growth substances merely aid in releasing the enzyme from the colloid. But just how the auxins are associated with the extensibility of the cell wall has remained until recently largely unknown. COMMONER, FOGEL, and MULLER (7) have experimented with the effect of auxin on water absorption by potato tuber tissue. Their results indicate that an auxin system regulates salt absorption processes in plant cells and through this effect influences water relations and cell enlargement.

Still another interpretation of cell elongation has been proposed by BURSTRÖM (3). In studies of the effects of indoleacetic on wheat roots, he differentiates two actions. The first is an accelerated rate of growth with increased wall plasticity before the start of elongation, and the second is an inhibition phase. "The action of indoleacetic is supposed to be a similar loosening of molecular joints during both phases of the elongation; during the first phase it increases

the swelling of the pectic (intermicellary) substances, during the second phase it prevents the deposition of cellulose micelles." He notes that roots recover from the inhibition, and may become adapted to even higher concentrations of indoleacetic.

The regulatory roles of growth substances just described may have been in large part responsible for the structural abnormalities of root hairs developed in indolepropionic solutions. While the hair cells developed no great lengths, their forked and greatly swollen apices indicated abnormal water relations, induced and greatly magnified by treatment with a high concentration of indolepropionic acid.

Some of the experiments described here were exploratory and the data obtained are presented despite the fact that they may not be wholly conclusive.

Summary

1. Since *Elodea* is an aquatic, excised bud-node and apical segments were readily grown in aqueous solutions of some sixteen growth substances and related compounds. The principal gross responses noted were axillary bud development, shoot elongation, the number of roots emerging, time and order of their appearance, their lengths, and root-hair development.

2. The injury of excision provided the initial stimulus for renewed growth in the bud nodes. Although some minor modifications were noted, where damage through treatment had not occurred, the general pattern of vegetative reproduction remained relatively unaltered in both control and test solutions. Excised bud nodes, in tap water alone, and cultured under suitable growing conditions, developed into complete new plants.

3. Nearly all the cultures were grown in 250-ml. glass tumblers under daylight fluorescent light with a daily light period of 13 hours. Experiments with different intensities of light showed that a reasonably good growth was maintained at 400 f.c.

4. In this species four root primordia are formed at each bud node very early in the ontogeny of the primary axis. There are two latent primordia in the primary axis in the region of divergence of the secondary axis, and two in the base of the secondary axis near the point of divergence from the primary axis. Even in rooted shoots as long as 1 m. these primordia remain latent. Upon excision of the bud node the two primary primordia emerge, and later the two secondary primordia may emerge. In control cultures in tap water these secondary primordia rarely appear, at least in the culture period of about 20 days. When these nodes are grown in solutions of certain indole or naphthalene compounds, stimulation of all four primordia may occur, and occasionally fifth roots may emerge.

5. For total root lengths at 16 days (experiment 1, concentration 1 p.p.m.) the decreasing magnitude of response was: indolepropionic, indolebutyric, indoleacetic, l-tryptophane, phenylacetic, control, B_1, and naphthaleneacetic. Average individual root length was: l-tryptophane, indolepropionic, control, phenylacetic, indoleacetic, B_1, indolebutyric, and naphthaleneacetic. For total number of roots the order was: indolebutyric, indolepropionic, indoleacetic, naphthaleneacetic (l-tryptophane, phenylacetic, and B_1 all similar), and control.

6. For total elongation of apical segments in the dark series at 129 hours (experiment 13, concentration 5 p.p.m.) the decreasing magnitude of response was: naphthaleneacetic, indolepropionic,

naphthalene acetamide, indolebutyric, indoleacetic, ascorbic, l-tryptophane (B_1 and histidine similar), and control. In the light series the order was the same through acetamide, and then the order was indoleacetic, indolebutyric, and control. The remainder of the substances had some inhibitory effect in the light. The highest growth rate measured was 0.30 cm./hr. (control, 0.13 cm./hr.) in the indoleacetic dark series at 21 hours.

7. Root hairs were rarely formed on the free-growing roots of excised bud nodes, larger segments, or shoots, growing under adequate light intensities. Root-hair formation did occur upon treatment with certain types of compounds. Of all the different types used, only those compounds with the indole or naphthalene ring system (and containing acid side chains) were associated with root-hair formation. In cultures in indolepropionic at 5 p.p.m., structural abnormalities developed in the terminal portions of the hair cells—possibly related to the abnormal water relations induced and greatly magnified by this high concentration. In view of the specificity of the response to certain types of compounds, this root-hair test of *Elodea* would appear to be a useful method of establishing a diagnostic character for the classification and characterization of certain of the growth-regulating substances.

8. The degree of inhibition and stimulation induced by many of the growth substances (aside from concentration) was a function of the light intensity, and to some extent the size of the segment employed. Increased growth of roots and shoots was associated with indolebutyric, indolepropionic, and histidine at low light intensities, where in control cultures little or no growth occurred. The association of growth-regulating substances with the synthesis of total solids, with starch hydrolysis, cellular respiration, salt absorption, and water regulation was considered in relation to cell elongation and to other growth processes.

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LITERATURE CITED

1. Bausor, S. C., Interrelation of organic materials in the growth substance response. Bot. Gaz. **103**: 710–724. 1942.
2. Borgström, G., Root hair formation as an auxin response. Proc. Roy. Phys. Soc. Lund **9**: 232–239. 1939.
3. Burström, H., The influence of heteroauxin on cell growth and root development. (Lantbrukshögskolans Annaler **10**: 209–240. 1942.) Biol. Abstr. **17**: 1972–1973. 1943.
4. Brown, W. H., The relation of the substratum to the growth of *Elodea*. Philippine Jour. Sci. (Botany) **8**: 1–20. 1913.
5. Caspary, R., Die Hydrilleen (Anacharideen Endl.). Jahrb. wiss. Bot. **1**: 377–513. 1858.
6. Clark, N. A., and Frahm, E. E., The effect of various growth-promoting substances on the reproduction of *Lemna*. Proc. Iowa Acad. Sci. **47**: 239–247. 1940.
7. Commoner, B., Fogel, S., and Muller, W., The mechanism of auxin action. The effect of auxin on water absorption by potato tuber tissue. Amer. Jour. Bot. **30**: 23–28. 1943.
8. Cormack, R. G. H., The development of root hairs by *Elodea canadensis*. New Phytol. **36**: 19–25. 1937.
9. Crocker, Wm., Hitchcock, A. E., and Zimmerman, P. W., Similarities in the effects of ethylene and the plant auxins. Contrib. Boyce Thompson Inst. **7**: 231–248. 1935.
10. Eyster, H. C., Auxin action. Science **97**: 358–359. 1943.
11. Holm, T., Recherches anatomiques et morphologiques sur deux monocotyledones submergées

(*Halophila baillonii* Asch. et *Elodea densa* Casp.). Bihang Svenska Vet.-Akad. Handlingar. **9**: 1–24. 1885.

12. Homès, M., and Van Schoor, G., La présence de substances de croissance chez *Elodea canadensis*. Bull. classe Sci. Acad. Roy. Belg. **23**:183–193. 1937.
13. Kraus, E. J., Histological reactions of bean plants to l-tryptophane. Bot. Gaz. **102**:602–622. 1941.
14. Mansfeld, R., Fedde. Repertorium **44**:292. 1938.
15. Mitchell, J. W., and Hamner, C. L., Stimulating effect of beta (3) indoleacetic acid on synthesis of solid matter by bean plants. Bot. Gaz. **99**:569–583. 1938.
16. Mitchell, J. W., Kraus, E. J., and Whitehead, Muriel R., Starch hydrolysis in bean leaves following spraying with alpha naphthaleneacetic acid emulsion. Bot. Gaz. **102**:97–104. 1940.
17. Mitchell, J. W., and Whitehead, Muriel R., Starch hydrolysis in bean leaves as affected by application of growth-regulating substances. Bot. Gaz. **102**:393–399. 1940.
18. Overbeek, J. van, Auxin in marine algae. Plant Physiol. **15**:291–299. 1940.
19. Rohrbaugh, L. M., Effects of light quality on growth and mineral nutrition of bean. Bot. Gaz. **104**:133–151. 1942.
20. ———, Unpublished data. University of Chicago. 1942.
21. Siddall, J. D., The American water weed, *Anacharis alsinastrum* Bab. Proc. Chester Soc. Nat. Sci. **1**:125–133. 1885.
22. Snow, Laetitia, The development of root hairs. Bot. Gaz. **40**:12–48. 1905.
23. Soltys, A., Umrath, K., and Umrath, Charlotte, Über Erregungssubstanz, Wuchstoff und Wachstum. Protoplasma **31**:454–480. 1938.
24. Weatherby, C. A., On the nomenclature of *Elodea*. Rhodora **34**:114–116. 1932.
25. White, P. R., Glycine in the nutrition of excised tomato roots. Plant Physiol. **14**:527–538. 1939.
26. Wilson, K., The production of root hairs in relation to the development of the piliferous layer. Ann. Bot. **50**:121–154. 1936.

EFFECTS OF OSMOTIC CONCENTRATION OF SUBSTRATE ON THE ENTRY OF WATER INTO CORN ROOTS

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Introduction

In a previous paper a potometric device for measuring the entry of water into roots was described, and quantitative data for corn and *Citrus* were reported (5). The studies with corn indicated that the rate of entry increases from a zone proximal to the root cap to a maximum at a point approximately 10 cm. from the root tip and that, in most cases, the rate decreases above this level. The studies reported here were initiated to determine the effect of the osmotic concentration of the substrate on entry of water, since this relationship has a bearing on problems encountered in irrigation agriculture where saline conditions exist. Supplementary experiments were undertaken to investigate the effect of removal of the shoot on entry of water into the roots.

Procedure

The experimental room was maintained at a temperature of 72° F. ±1, with a relative humidity of 40% ±5. Overhead illumination was supplied by Westinghouse white fluorescent tubes. The room was equipped with six aquaria, and six plants were used in each experimental run (fig. 1).

Mexican June corn was used as the test plant. The seeds were germinated in sphagnum and the seedlings transferred to aerated water cultures containing a complete nutrient solution. The young plants were grown in the greenhouse until they were 10–12 inches tall, with five or six expanded leaves. At this stage they had a cycle of several adventitious roots which exceeded 10 cm. in length, with a diameter of 1.1–1.8 mm. Uniform sets of plants were selected at 8:00 A.M., the installation and potometric adjustments were completed by 10:00 A.M., and half-hourly readings were made until 3:30 P.M. As in the initial studies (5), it was found desirable to excise all but the test root at the beginning of the experiment, since this helped to eliminate variations due to differences in total absorbing surface. When all roots are left intact, the test root exhibits the same type of gradient as under these conditions but the rate of entry is appreciably slower. At the conclusion of each experimental run the root segments inclosed in the potometric chambers were measured with micrometer calipers and their area determined. Test plants were discarded when there was evidence of injury due to technical difficulties in attaching the potometers.

Three substrates were used: (*a*) the base nutrient at 0.8 atm. osmotic concentration, (*b*) the base nutrient plus added NaCl (48 m.e./l.) to produce 2.8 atm., and (*c*) the base nutrient plus NaCl (96 m.e./l.) to produce 4.8 atm. The actual osmotic concentrations were calculated from freezing-point depression determinations. In order to study the effect of the status of the test root on rate of entry of water, plants were preconditioned in one series of experiments. The concentration of the nutrient solution in which the plants were grown was increased by steps of 0.5 atm. per day to the desired level (2.8 or 4.8 atm.) by adding successive increments of NaCl. The plants were maintained at the final concentration for 5–7 days to permit the roots to attain the required length and become conditioned to the final osmotic concentration of the substrate.

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Investigation

A. Nonconditioned plants

Following the procedure used in the initial experiments (5), roots of nonconditioned plants were tested at the 10-, 6-, and 2-cm. levels in substrates with osmotic concentrations of 0.8, 2.8, and 4.8 atm., respectively. In all substrates the rate of entry was lowest at the 2-cm. level, and in the control (base nutrient) treatment there was a significantly descending gradient of rate of entry from the 10- to the 2-cm. levels in approximately 82% of the plants tested (table 1). Under the 2.8 and 4.8 atm. treatments there was little difference in rate between the 10- and 6-cm. levels, but in both cases it fell sharply at the 2-cm. level. On the basis of the data obtained, it appeared that in practically all cases the zone between the 10- and 6-cm. levels could be regarded as the region of maximum entry of water. For this reason the average of the rates of entry for these two levels under any given treatment was used in some comparisons as being representative of the region of most active absorption (table 2).

When the rates at corresponding root levels under the three treatments are compared, it is evident that increased concentration of salts in the substrate results in a very significant decrease in entry of water (fig. 2). The reduction at

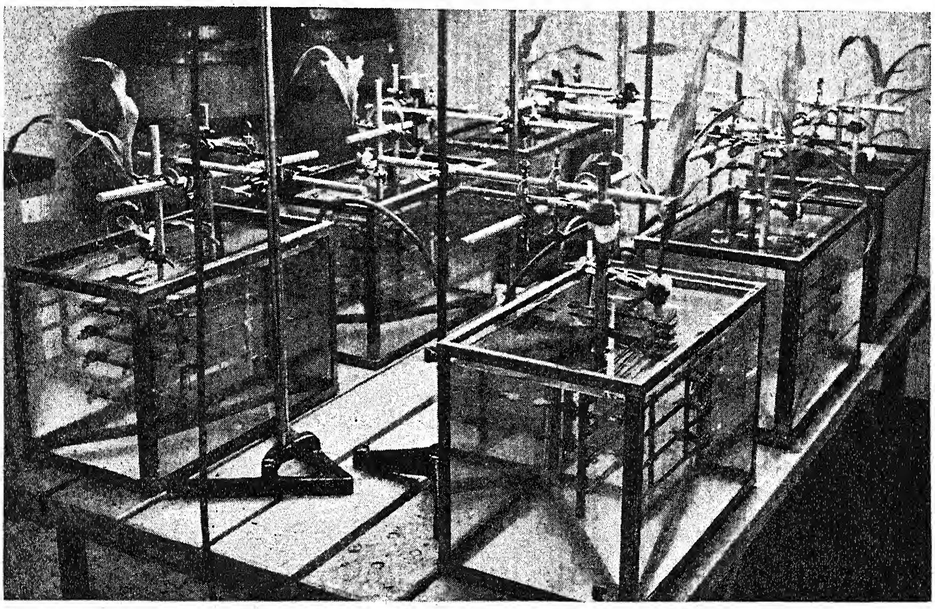


Fig. 1.—Aquaria with test plants in position. Three potometers attached to each test root at 2, 6, and 10 cm. from root tip; fourth potometer is the check.

TABLE 1

Location of maximum rate of entry of water

Treatment	Maximum rate	
	At 10-cm. level (%)	At 6-cm. level (%)
Base nutrient (control)....	82	18
2.8 atm. nonconditioned..	62	38
2.8 atm. preconditioned...	59	41
4.8 atm. nonconditioned...	53	47
4.8 atm. preconditioned...	33	67

TABLE 2

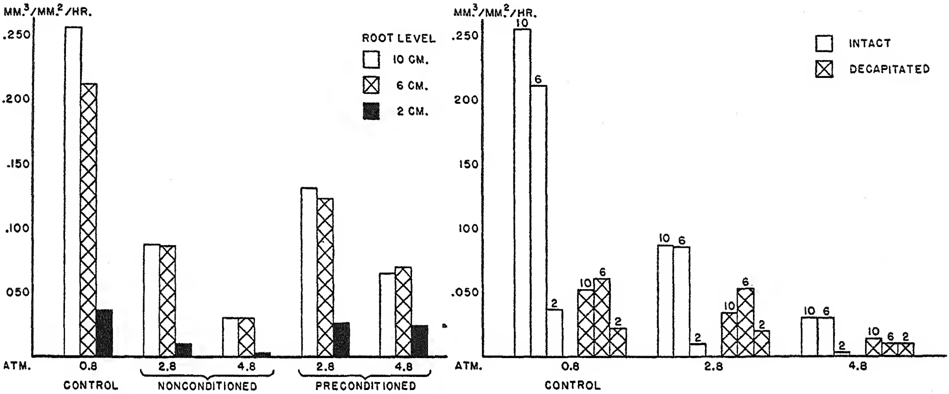
MEAN RATE OF ENTRY OF WATER AND PERCENTAGE DECREASE IN RATE FOR NONCONDITIONED AND PRECONDITIONED ROOTS

ROOT LEVEL	TREATMENT AND OSMOTIC CONCENTRATION										
	Control (base nutrient) 0.8 atm. os. conc.	Base nutrient + 2 atm. NaCl (total os. conc., 2.8 atm.)				Base nutrient + 4 atm. NaCl (total os. conc., 4.8 atm.)					
	Nonconditioned	Nonconditioned		Preconditioned		Nonconditioned			Preconditioned		
	Entry (mm.³/mm.²/hr.)	Entry (mm.³/mm.²/hr.)	Percentage decrease from control	Entry (mm.³/mm.²/hr.)	Percentage decrease from control	Entry (mm.³/mm.²/hr.)	Percentage decrease from 2.8 atm.	Percentage decrease from Control	Entry (mm.³/mm.²/hr.)	Percentage decrease from 2.8 atm.	Percentage decrease from Control
10 cm.	0.249(19)*	0.075(12)	70	0.102(14)	59	0.030(15)	60	88	0.065(18)	36	74
6 cm.	.211(20)	.086(13)	59	.123(17)	42	.023(13)	73	89	.066(17)	46	69
2 cm.	.038(25)	.010(12)	74	.024(22)	37	.003(13)	70	92	.024(18)	0	37
Ave. 10 cm. + 6 cm.	0.229(39)	0.082(25)	64	0.114(31)	50	0.027(28)	67	88	0.065(35)	43	72

* Figures in parentheses indicate number of roots tested.

each higher level of concentration ranges from 59 to 74%. If the average of the rates at the 6- and 10-cm. levels is used as a basis of comparison, the percentage decrease under the treatments at 2.8 and 4.8 osmotic concentrations is 64% and 67%, respectively. At the highest salt level the rate of entry of water into the root is reduced to 12% of that absorbed under the control treatment (table 2).

ditioned plants, the rate at corresponding root levels was lower in substrates of high osmotic concentration, but the decrease in rate of preconditioned plants was significantly less than that for nonconditioned ones, indicating that the plant has some capacity to adjust itself to high concentrations of salt (10). At 2.8 atm. osmotic concentration the average of the rates for the 6- and 10-cm. levels was reduced 50% from the control rate, as compared with 64% with nonconditioned plants. At 4.8 atm. concentration the rates for preconditioned and nonconditioned plants were 43 and 67%, respectively, lower than the 2.8 rates; and the rate for preconditioned plants was 72% less than the controls as compared with 88% for the nonconditioned plants (table 2).



FIGS. 2, 3.—Fig. 2 (left), rate of entry of water into adventitious roots of corn at three levels in substrates of 0.8, 2.8, and 4.8 atm. osmotic concentration. Fig. 3 (right), effect of decapitation on entry of water into corn roots at 10-, 6-, and 2-cm. levels in substrates of 0.8, 2.8, and 4.8 atm. osmotic concentration.

B. Preconditioned plants

Except during the period of germination and seedling development, plants grown in saline soils under irrigation are not ordinarily subjected to such drastic changes in concentration of the substrate as occur when nonconditioned plants are transferred from a nutrient solution of 0.8 atm. osmotic concentration to one at 2.8 or 4.8 atm. For this reason plants were preconditioned as described in the section on Procedure, and the entry of water was determined in the same manner as for nonconditioned plants.

With preconditioned plants there was little difference in rate of entry at the 6- and 10-cm. levels but a sharp drop at the 2-cm. zone (fig. 2). Like the noncon-

With preconditioned plants in the 4.8 atm. substrate, 67% of the test roots had somewhat higher rates of entry at the 6-cm. than at the 10-cm. level (table 1). This shift in the axial gradient of rate of entry appears to be due in part to the relatively advanced maturity at corresponding test levels of the roots precon-

ditioned in substrates of high salt concentration and in part to the concentration of the substrate itself.

It has been pointed out that variations in relative maturity due to differences in rate of growth and in the nutritional status of test plants may materially affect the rate of entry of water and salts (5, 7, 13). Histological considerations relative to this situation are discussed in a later section. Although all test plants were grown and preconditioned in the same manner, some differences in their nutritional status resulted from variation in climatic factors, since the studies were carried on during a period of several months.

Because of these and other variables incident to the use of living plants as the test units, the data were subjected to statistical treatment. The homogeneity of the results obtained under each treatment was tested, and a pooled analysis of variance, set up to account for disproportionate frequencies in number of observations, was carried out. The analysis based on the combined average rates of the 6- and 10-cm. levels was computed for the ten possible individual comparisons that can be made between the five treatments. Highly significant differences were found in all but two cases. The difference between the nonconditioned and the preconditioned 2.8 atm. treatments was significant, but there was no significant difference between the nonconditioned 2.8 atm. and the preconditioned 4.8 atm. treatments.

C. Decapitated plants

Investigations of entry of water into roots have led to a distinction between the "active suction" of the root system itself and the "passive suction" transmitted to the roots from the shoot (9, 10). Some quantitative studies have been made dealing with the role of the root, per se, in absorption. Rosene (14), using intact and excised onion roots, found that "the rates of water influx at contiguous levels of the same root after excision were equal to, greater, or less than before excision." Kramer (9) found that "the rate of exudation from detopped root systems was only one to five per cent of the rate of transpiration from similar intact plants." White (18), on the other hand, working with excised tomato roots in vitro, demonstrated that root pressure may be an important factor in sap movement, exceeding 6 atmospheres external pressure.

In order to compare the rate of water entry into roots of intact with that of decapitated plants, the tops were excised just above the level at which the adventitious roots emerge. This was done after the potometers were attached and all adjustments had been made. The decapitated plants were then tested in the same substrates as used in the preceding experiments. The quantitative data are presented in table 3.

The results following decapitation indicate a marked reduction in entry rate at all concentrations of the substrate at the 10- and 6-cm. root levels (fig. 3). At both levels there was a greater reduction in the base-nutrient solution than in the substrates of higher osmotic concentration. At all root levels the rate is reduced in both intact and decapitated plants as the concentration of the substrate is increased, but the reduction is much less with the latter. In general, it appears that the lower the rate of entry into the intact plant, the less percentile reduction in rate results from its decapitation. The tendency toward uniformity in rates of entry into intact and decapitated plants at the 2-cm. level suggests that entry at that level is due primarily to the "active"

TABLE 3

EFFECT OF DECAPITATION ON ENTRY OF WATER INTO ROOT

Treatment and osmotic concentration of substrate	Water entry (mm.³/mm.²/hr. at three root levels)						Average of 10 and 6 cm.	
	10 cm.		6 cm.		2 cm.			
	Intact	Decapitated	Intact	Decapitated	Intact	Decapitated	Intact	Decapitated
Control (base nutrient) 0.8 atm.	0.249(19)*	0.052(12)	0.211(20)	0.061(12)	0.038(25)	0.022(12)	0.229(39)	0.057(22)
Base nutrient + NaCl 2.8 atm.	.075(12)	.034 (7)	.086(13)	.053 (7)	.010(12)	.020 (7)	.081(25)	.044(14)
Base nutrient + NaCl 4.8 atm.	0.030(15)	0.014(12)	0.023(13)	0.010(13)	0.003(13)	0.010(13)	0.027(28)	0.012(23)

* Figures in parentheses indicate number of roots tested.

absorption of the root; and that, regardless of the concentration of the substrate, there is a relatively constant and rather low water requirement for the apical portion of the root.

ANATOMY AND HISTOLOGY OF CORN ROOT

Mature adventitious roots of corn consist of a central stele, cortex, and epidermis (1, 4). As seen in transection, the stele has a ring of twenty to forty protoxylem strands alternating with small groups of phloem (fig. 4). There are two or three protoxylem strands to each large metaxylem vessel, and the latter are adjacent to the central pith. The outermost cells of the pith extend between the xylem elements and become thick-walled and lignified at maturity, forming a continuous zone of connective tissue. The outermost layer of the stele is the pericycle, which consists of thin-walled cells.

The cortex is comprised of several layers of parenchymatous cells, the innermost one forming the endodermis. The endodermal cells abut those of the pericycle, and at maturity all (except the passage cells which occur on the same radii as the protoxylem points) develop a U-shaped type of thickening on the radial, end, and inner tangential walls (fig. 5). All the endodermal cells have

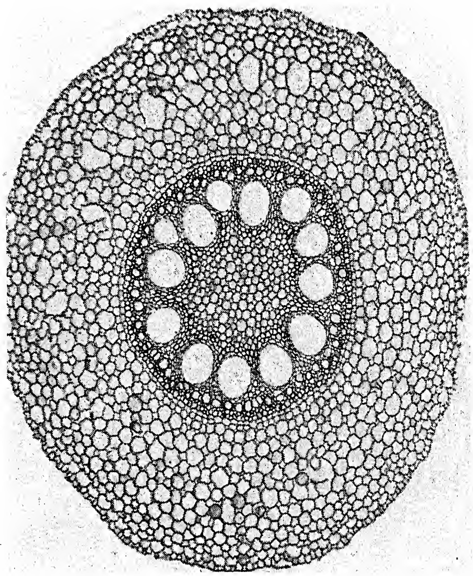


FIG. 4.—Transection of adventitious root of corn at 6 cm. from root tip showing general organization of stele, cortex, and epidermis. Large metaxylem vessels not fully mature but have attained maximum size and are surrounded by lignified connective cells.

Casparian strips, which are more obvious before secondary wall thickening has occurred. The epidermis which forms the

surface layer of the root is ephemeral, disintegrating as the root matures; and the outer layers of the cortex form a lignified and suberized hypodermis.

Microchemical and histological studies of test roots at the 10-, 6-, and 2-cm. levels were made to determine to what extent the observed gradient of entry of water could be correlated with the anatomical status of the root. Freshly cut sections were compared with others primordia. The root hairs are shorter and less numerous when the plants are grown in water culture. This should be borne in mind when applying these data to plants growing in soil. Under field conditions, the development of root hairs would be at a peak in the region of differentiation and maturation (6–10-cm. zone), and this would tend to accentuate the gradient of entry of water observed in water-culture studies.

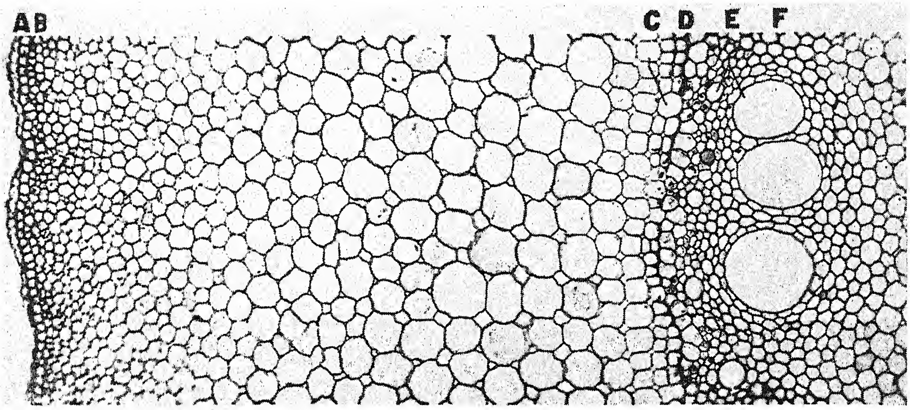


FIG. 5.—Transection of mature corn root showing *A*, disintegrating epidermal layer; *B*, lignified hypodermal cells; *C*, passage cells of endodermis; *D*, endodermal cells with U-shaped thickenings; *E*, mature vascular elements of stele; and *F*, zone of lignified connective tissue.

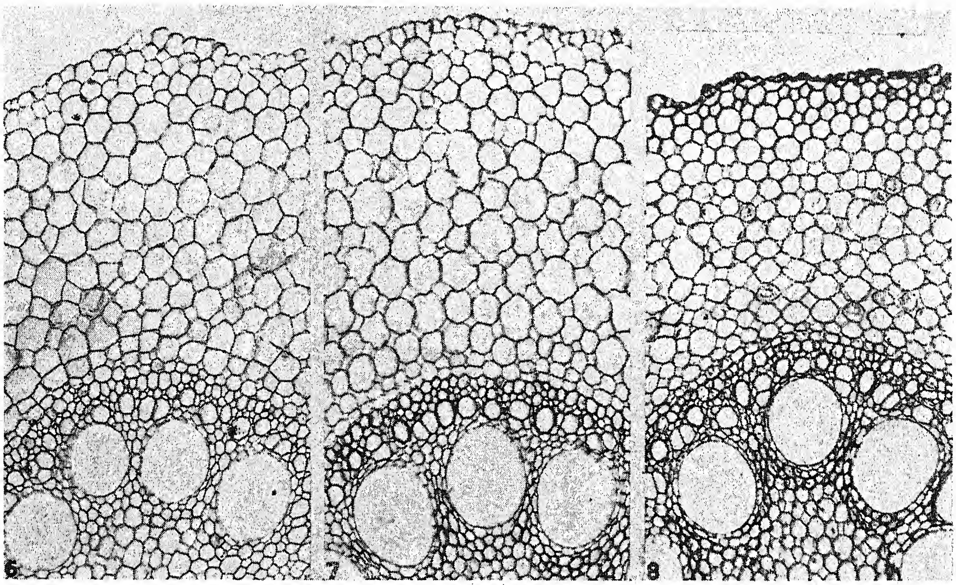
that had been fixed, infiltrated with paraffin, and stained with Flemming's triple stain. With the fresh sections Sudan III was used for suberin, phloroglucin-HCl and $KMnO_4$ for lignin, and chlor-zinc-iodide and cuprammonia for cellulose. Resorcin blue gave good differentiation of the Casparian strips.

The progressive development of the tissues of the corn root is illustrated in figures 6–8. The histological changes of most interest in relation to entry of water are those occurring in the vascular, endodermal, and hypodermal tissues. The epidermis does not undergo much change between the 2- and 10-cm. levels, except that there is some elongation of root-hair

At the lowest level (2 cm.) at which entry of water was measured, the functional protoxylem elements have thin lignified walls, while the metaxylem vessels are still in process of differentiation, as evidenced by the presence of cytoplasm and nuclei in the vessel segments (fig. 6). The end walls of these segments are still present, so that the large vessels do not function in water transport except as there may be a limited segment-to-segment movement (fig. 9*B*). Under special staining, Casparian strips can be observed on the radial and end walls of the endodermal cells.

At the intermediate level (6 cm.) there is evidence of further lignification of the

protoxylem and advanced maturation of the outermost metaxylem elements. Most of the large innermost metaxylem vessels are probably functional at this level, as the end walls of the vessel segments are partially or wholly resorbed in a majority of cases observed. The radial walls of the hypodermis may show initial suberization, and the Casparian strips of the endodermis are suberized and give radial walls of the endodermis are suberized and partially lignified, but all the endodermal cells are still "passage cells" as far as structure is concerned. Suberization and lignification of the inner tangential walls do not occur until the root is more nearly mature (fig. 5). The walls of some of the hypodermal cells are suberized, and there are indications of the deposition of lignin-like substances.



FIGS. 6–8.—Transections of nonconditioned corn roots showing relative maturation of hypodermal, endodermal, vascular and connective tissues at 2-cm. (fig. 6), 6-cm. (fig. 7), and 10-cm. level (fig. 8). Root photographed at 10-cm. level was slightly smaller in diameter than those shown in figs. 6 and 7. All at same magnification.

some indication of the deposition of a lignin-like substance. A limited number of well-developed root hairs occur (fig. 7).

Vascular differentiation is well advanced at the highest level (10 cm.) of measurement (fig. 8). The protoxylem elements and the metaxylem vessels are completely mature (fig. 9*A*). The maturity of the stele is indicated further by the thick-walled medullary parenchyma, which forms a zone of connective tissue adjacent to the metaxylem vessels. The

The relative maturity of the root at a given level will be modified by the environmental conditions existing during the growing period. When conditions favor rapid growth and elongation, the 10-cm. level will be less mature than when conditions are less favorable and the growth rate is slow. This difference in growth rate creates a variable in test roots, which accounts in part for variations in rate and gradient of water entry under a given set of experimental conditions.

The effect of the concentration of the substrate on rate of maturation of corn roots is evident when they are preconditioned by additions of NaCl to the nutrient solution. If the plants are conditioned in a substrate of 2.8 atm. osmotic concentration, the roots reach the required length more slowly than those grown in nutrient solution at 0.8 atm. concentration, and more pronounced growth inhibition is observed when plants are conditioned to an osmotic concentration of 4.8 atm. In general, it took the plants preconditioned to the 4.8 level 2–4 days longer to produce roots of suitable length for the tests. The diameters of nonconditioned and preconditioned roots at the test levels were not significantly different, averaging 1.33–1.37 mm.

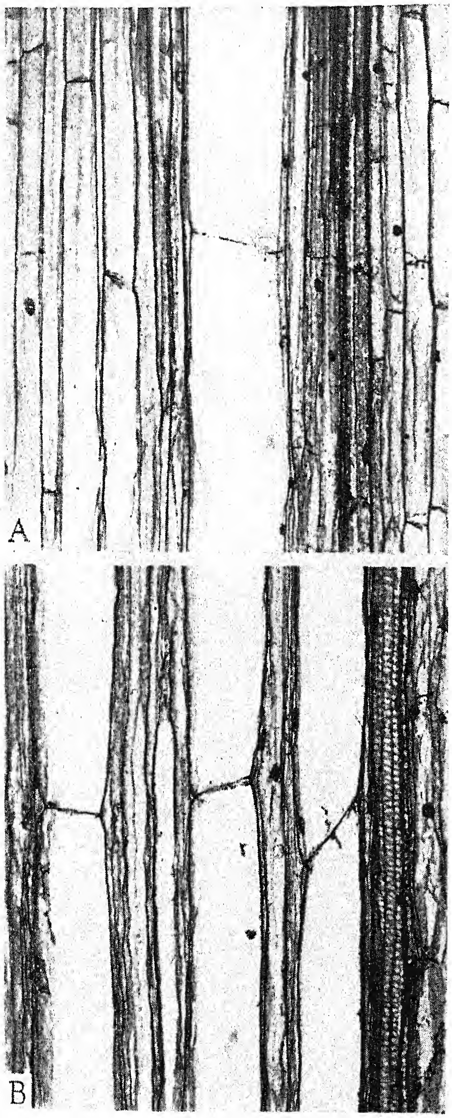


FIG. 9.—Tangential longisections of corn root cut through large metaxylem vessels. *A*, at 10-cm. level; *B*, at 2-cm. level. In *B*, metaxylem vessels are still immature and end walls of vessel segments intact. In *A*, metaxylem vessel has attained full size and end wall of vessel segment almost completely resorbed. Walls of adjacent connective tissue are lignified. Both at same magnification.

Histological studies at comparable levels indicated that the slower growth rate of the preconditioned roots is reflected in the more advanced maturation of the tissues as compared with those of nonconditioned roots. At the 2-cm. level no significant differences in relative differentiation of tissues could be noted between nonconditioned and preconditioned roots; at the 6- and 10-cm. levels the tissues were more mature in the latter. At the 6-cm. level the vascular elements and the connective tissue surrounding them were slightly thicker-walled and more lignified than those of the nonconditioned roots. No outstanding differences in the endodermal and hypodermal tissues were observed.

At the 10-cm. level the preconditioned roots were much more mature than the nonconditioned ones. The secondary walls of the metaxylem elements were thicker, there was more lignification of the endodermis, and suberization of the walls of the hypodermal cells was more advanced. This latter condition may account in part for the shift in the peak of the gradient of water entry to the 6-cm. level which was observed in 67% of the

preconditioned roots at the 4.8 atm. concentration (table 1).

Discussion

The entry of water into roots and its transport to the stele have been ascribed to an osmotic mechanism, to imbibition, to secretory action, or to some combination of such forces. In any event, work must be done and energy supplied to do that work. HENDERSON (6), using corn seedlings in liquid cultures, found a close correlation between the volume of water absorbed and the respiration of the root, indicating an expenditure of energy in the process of absorption. VAN OVERBEEK (17), experimenting with decapitated tomato plants, described an "active" component of root pressure that could be reversibly eliminated by KCN and suggested that it was connected with respiratory processes. GREGORY and WOODFORD (3) found that oxygen intake was greatest in the segment containing the tip of the root of *Vicia faba* and decreased in the next two segments from the tip, but they were not able to demonstrate consistent differences in water uptake in the different segments.

In the present studies with nonconditioned plants, an ascending axial gradient in the rate of entry of water was observed in a great majority of cases when rates at the 2-, 6-, and 10-cm. root levels were compared. Preconditioning, with the consequent retardation in rate of growth, tended to shift the point of most rapid entry toward the apical region of the root, so that frequently the highest rate was recorded at the 6-cm. level. A similar shift occurred owing to high concentration of the substrate in many cases. In all cases the rate was lowest at the 2-cm. level, and the general picture is in agreement with our earlier study (5) and that of ROSENE (13).

The major portion of the water required by a plant enters the root in a zone extending from the level at which differentiation of the xylem elements occurs to the level at which the endodermis becomes entirely suberized (11, 15, 16). But the rate of entry is not equal throughout this zone, and in the adventitious roots of corn the observed rates were well correlated with the relative development of the xylem at the three test levels. At the 2-cm. level, where the lowest rates of entry occurred, only the first protoxylem elements were mature; at the 6-cm. level, where the intermediate metaxylem vessels were also fully differentiated, higher rates of entry occurred. In most cases the largest metaxylem vessels were fully mature at approximately the 10-cm. level, at which point the highest rates were recorded, except in instances where growth in length was retarded by preconditioning in a saline substrate or by unfavorable climatic conditions.

A second limiting histological factor is the status of the cortical and epidermal tissues with respect to suberization and lignification. At the apex of the root the root-cap cells are impermeable to water, but this zone is not more than a few millimeters in extent. PRIESTLEY and TUPPER-CAREY (12) have found that the meristematic tissue at the root apex is relatively impermeable to water under normal conditions. It seems probable that the water requirements of the root apex are less than those of the zone of elongation, which—in addition to requiring hydration for growth—is (together with the zone of maturation above it) the region through which water must be transported to meet the requirements of the shoot.

Above the level of most rapid entry the limiting factor appears to be the suberization of the hypodermis and endodermis. In the corn root there is a progressive suberization of the hypodermal layers. At approximately 4 cm. from the root tip the thin radial and end walls of the hypodermis may be slightly suberized; at the 10-cm. level there is marked suberization of these walls, and in some cases suberization of the inner tangential walls has begun. At higher levels all the walls of the hypodermis—and some of those of the subhypodermal cells—are suberized, and there is some lignification. Paralleling this development, the walls of the endodermal cells become suberized and impregnated with a lignin-like substance. Casparian strips are evident at the 2-cm. level. There are indications of definite suberization and deposition of lignin-like substances on the Casparian strips at the 6-cm. level, which become more pronounced toward the 10-cm. level. Above this point suberization and lignification of the inner tangential walls begin, but the tangential walls of some of the passage cells remain unsuberized, permitting a limited cross-transfer of water.

Thus the histological picture of root ontogeny is correlated with the gradient of water entry, although this gradient may be modified by variations in the rate of growth of the root and the rate of maturation of the vascular, cortical, and epidermal tissues. However, roots of different species exhibit marked differences in their development (4), and it is to be expected that gradients of other types may be found when quantitative measurements of entry are made.

There are few quantitative data that indicate the extent to which the entry of water is restricted when the osmotic concentration of the soil water or nutrient solution is high. EATON (2), using a divided root system technique, found that the entry of water into corn and tomato roots was progressively reduced by adding increments of 10, 20, and 40 m.e./l. Cl. Using nutrient solutions of 0.3 and 1.8 atm. osmotic concentration, he found that the entry of water into corn roots was twice as rapid in the solution of lower osmotic pressure.

Our data based on direct measurement of water entry per unit of surface area per hour indicate a pronounced reduction in rate at all levels when the concentration of the substrate is increased. With nonconditioned plants an increase of 4 atm. in osmotic concentration reduced the rate 88%. When the test plants were gradually preconditioned to the higher osmotic concentrations over a period of several days, the rate of entry was 16% greater than with the nonconditioned plants, indicating some adaptive capacity in this regard.

Salt-tolerance tests at the United States Regional Salinity Laboratory and elsewhere (8) indicate that corn is less tolerant to high salt concentration than many other crop plants. It seems probable that the more salt-tolerant species would show a less pronounced response to high concentration of the substrate in terms of reduced water entry than did corn. It is possible that the relative tolerance of crop plants to saline conditions may depend in large measure upon the rate of water entry when the soil solution has a high osmotic concentration.

The marked reduction in rate of entry which follows decapitation emphasizes the role of the shoot in this process. The loss of water from the shoot by transpiration and its utilization in synthetic processes in the leaves are major factors in determining the rate of entry into the root.

The root of a decapitated plant, through its osmotic mechanism, imbibition, and the expenditure of energy, may obtain water even when the osmotic concentration of the substrate is high, but the rate of entry is low compared with that of an intact plant.

There is much less difference in the rates of entry at the three test levels in decapitated than in intact plants. The difference in rate at the 10-, 6-, and 2-cm. levels with decapitated plants in the base nutrient and the 2.8 atm. solutions is not great and is practically negligible at the 4.8 concentration. The somewhat higher rates at the 10- and 6-cm. levels are probably due to the movement of water to the upper portion of the root and the basal portion of the stem from which the adventitious roots arise. The values obtained at the 2-cm. level indicate that the tips of roots of decapitated and intact plants take in essentially no more water than is utilized locally.

Summary

The entry of water into corn roots was determined quantitatively with a potometric device attached at three root levels: 2, 6, and 10 cm. from the root tip. Substrates of three osmotic concentrations were used—0.8 (control), 2.8, and 4.8 atm., with NaCl as the salt added to the base nutrient solution. Tests were run with nonconditioned, preconditioned, and decapitated plants.

1. In general the most rapid entry occurred in the zone between the 6- and 10-cm. levels above the root tip. In all tests the rate of entry was lowest at the 2-cm. level, and 82% of the control plants exhibited the highest rate at the 10-cm. level.

2. The state of maturity of the vascular tissue affects the rate of entry. The differentiation of the water-conducting elements is progressive, and the full complement of vascular tissue is not functional at points below the level of most rapid entry.

3. The rate of entry increases as the epidermal cells mature. It decreases as they disintegrate and the hypodermal and endodermal cells become suberized and lignified.

4. Substrates of high osmotic concentration tend to inhibit meristematic activity and elongation of the root. Under these conditions the zone of most rapid entry is nearer the root tip.

5. With both nonconditioned and preconditioned plants, high osmotic concentration of the substrate resulted in a significant reduction in the rate of entry.

6. At corresponding root levels, and in substrates of equal osmotic concentration, preconditioned plants had higher rates than nonconditioned ones.

7. When compared with intact plants, decapitated ones exhibited a marked reduction in entry rate in all substrates and at all root levels except the 2-cm. level in the high salt solutions.

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LITERATURE CITED

1. Avery, G. S. Jr., Comparative anatomy and morphology of embryos and seedlings of maize, oats, and wheat. Bot. Gaz. **89**:1–39. 1930.
2. Eaton, F. M., Water uptake and root growth as influenced by inequalities in the concentration of the substrate. Plant Physiol. **16**:545–564. 1941.
3. Gregory, F. G., and Woodford, H. K., An apparatus for the study of the oxygen, salt, and water uptake of various zones of the root with some preliminary results with *Vicia faba*. Ann. Bot. (N.S.) **3**:146–154. 1939.
4. Hayward, H. E., The structure of economic plants. Macmillan Co., New York. 1938.

5. HAYWARD, H. E., BLAIR, WINIFRED M., and SKALING, P. E., Device for measuring entry of water into roots. BOT. GAZ. 104:152–160. 1942.
6. HENDERSON, LETA, Relation between root respiration and absorption. Plant Physiol. 9:283–300. 1934.
7. HOAGLAND, D. R., and BROYER, T. C., General nature of salt accumulation by roots with description of experimental methods. Plant Physiol. 11:471–507. 1936.
8. KEARNEY, T. H., and SCOFIELD, C. S., The choice of crops for saline land. U.S.D.A. Circ. 404. 1936.
9. KRAMER, P. J., The force concerned in the intake of water by transpiring plants. Amer. Jour. Bot. 26:784–791. 1939.
10. MAXIMOV, N. A., The plant in relation to water. Allen and Unwin, London. 1929.
11. POPESCO, ST., Recherches sur la région absorbante de la racine. Bull. Agr. Bucarest 4:59–191. 1926.
12. PRIESTLEY, J. H., and TUPPER-CAREY, R. M., Physiological studies in plant anatomy. IV. The water relations of the plant growing point. New Phytol. 21:210–229. 1922.
13. ROSENE, HILDA F., Distribution of the velocities of absorption of water in the onion root. Plant Physiol. 12:1–19. 1937.
14. ———, Comparison of water intake in contiguous regions of intact and isolated roots. Plant Physiol. 16:19–38. 1941.
15. SCOTT, LORNA I., The root as an absorbing organ. II. The delimitation of the absorbing zone. New Phytol. 27:141–174. 1928.
16. SCOTT, LORNA I., and PRIESTLEY, J. H., The root as an absorbing organ. I. A reconsideration of the entry of water and salts in the absorbing region. New Phytol. 27:125–140. 1928.
17. VAN OVERBEEK, J., Water uptake by excised root systems of the tomato due to non-osmotic forces. Amer. Jour. Bot. 29:677–683. 1942.
18. WHITE, P. R., "Root pressure"—an unappreciated force in sap movement. Plant Physiol. 25:223–227. 1938.

GROWTH AND DEVELOPMENT IN RANGE GRASSES. III. PHOTO-PERIODIC RESPONSES IN THE GENUS BOUTELOUA[1]

CONTRIBUTIONS FROM THE HULL BOTANICAL LABORATORY 556

CHARLES E. OLMSTED

Introduction

Since only limited studies (4, 16) on photoperiodic responses of native range grasses have been made, in contrast with more extensive work on cultivated forage grasses and cereals (2, 3, 6, 7, 8, 17, 23), and because there were indications (4, 16, 18, 21, 25) that some species in the genus *Bouteloua* might be photoperiodically sensitive, it was felt that such an investigation of this genus might be timely.

The genus is of great economic importance in the western half of the United States, where several of its species contribute largely to the forage supply on many native ranges, and where some of them show great promise in artificial reseeding of denuded range lands (11, 13, 24, 27). It is a rather sharply defined—although diverse—taxonomic unit, the species falling into two subgenera (11, 12). The various species show considerable differences in distribution and in adaptation to environment. Recent investigations (10, 14, 15, 19, 21, 22, 24) have emphasized a morphological, physiological, and cytological diversity within certain individual species. Some of these facts suggested that a knowledge of photoperiodic responses in the genus might contribute to an understanding (*a*) of the factors limiting the geographical distribution of the different species, and (*b*) of those affecting vegetative and reproductive habits during the growing season, both for the species under investigation and for the grass life-form in general. Such knowledge (*c*) should be of value in showing whether photoperiodic requirements need be considered in breeding programs aimed to produce desirable strains for reseeding and (*d*) might contribute to further understanding of the evolution of photoperiodic responses within taxonomic units.

Experiments were initiated in the spring of 1941 at the University of Chicago and have been continued since. These experiments were the outgrowth of earlier ones conducted at the University of Arizona on drought responses of *B. curtipendula*, in which certain unexpected results were obtained in plants grown with supplemental illumination during the winter months (18). The present paper is concerned with the photoperiodic responses of one strain each of six species of the genus, as shown by survey experiments conducted in 1941 and 1942. On the basis of these results, other experiments, now in progress (15, 19), were initiated along more specific lines. They will be reported on at a later date.

Material, methods, and environmental conditions

The six species investigated in 1941 were side-oats grama (*Bouteloua curtipendula*), slender grama (*B. filiformis*), hairy grama (*B. hirsuta*), Rothrock's grama (*B. rothrockii*), black grama (*B. eriopoda*), and blue grama (*B. gracilis*). The first two species are in the subgenus Atheropogon, and the others in the subgenus Chondrosium. Spikelets of the first three species, collected on the Santa Rita Experimental Range in southern Arizona, were obtained through the courtesy of the Southwestern Forest and Range Experiment Station and Dr.

[1] This work was aided in part by a grant from the Dr. Wallace C. and Clara A. Abbott Memorial Fund of the University of Chicago.

ROBERT DARROW of the University of Arizona. Caryopses of Rothrock's grama from Sells, Arizona, and of black grama from Pima, Arizona, were obtained through the kindness of Mr. L. P. HAMILTON of the Soil Conservation Service, Tucson; while Mr. LEON HURTT of the Northern Rocky Mountain Forest and Range Experiment Station, Missoula, Montana, furnished caryopses of blue grama from Silver Star, Montana. Thus material of five of the six species originated within a fairly small region in southern Arizona under similar natural photoperiods, while that of blue grama came from a source about 13° in latitude north of the others and thus from an area with considerably different natural photoperiodic conditions.

Side-oats, hairy, and blue grama are species of wide distribution in western United States, extending south into Mexico or even farther. Blue and side-oats grama range into Canada, and hairy grama occurs in South Dakota. Over their entire ranges these species are subjected to widely differing conditions of maximum day length in the growing season, especially when contrasted with the other three species (slender, black, and Rothrock's grama), which occur in the United States only in southern New Mexico, Arizona, southwestern Texas, or southern California, extending south into Mexico or South America (11, 12).

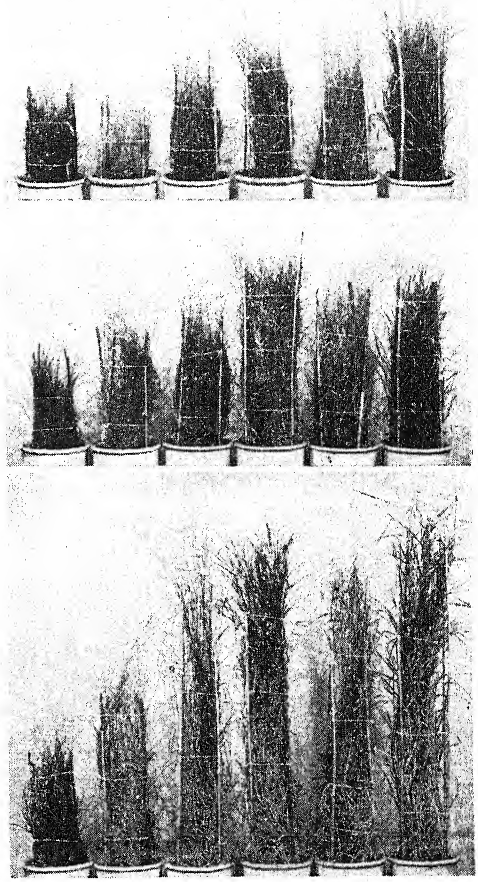
One hundred eighty 2-gallon glazed crocks, 8 inches in diameter and $10\frac{1}{2}$ inches deep (inside measurements), with rounded bottoms provided with a center drain, were uniformly spaced on six trucks (30 per truck) which could be rolled into ventilated lightproof photoperiodic sheds. The crocks were evenly filled and packed with a sandy loam soil of prairie origin (near Chicago) and of medium fertility. It proved to be favorable for all six species. On April 6, 1941, uniform caryopses were planted 1 cm. deep at evenly spaced intervals at the rate of 25–35 per crock (150–200 for *B. rothrockii*). Thirty pots were planted to each species, five on each of the six trucks. To compensate for possible positional effects, the crocks were at first systematically distributed on the trucks, but it later proved necessary to arrange them so that the taller species did not shade the shorter ones. Positional effects were found not especially important.

Immediately after prompt and uniform (except in *B. eriopoda*) germination, the plants on the three trucks in each of the two adjoining rooms were subjected to photoperiods of 8, 12, and 16 hours. Trucks on 8- and 12-hour photoperiods received natural daylight between 8:00 A.M. and 4:00 P.M. (CDT) each day in the greenhouse but were rolled into the lightproof sheds for the remainder of the 24 hours. Trucks on 12-hour photoperiod also received 4 hours of artificial light from 4:00 to 8:00 P.M. Trucks on 16-hour photoperiod remained in the greenhouses continuously and received supplementary illumination after sunset throughout the season. Supplementary illumination over each truck was provided by three 200-watt Mazda lamps mounted in individual reflectors. Light intensity from this source at pot-level varied from 100 to 180 foot candles (Weston Sunlight meter), depending on position and plant height. The lights were raised as the plants grew to maintain this intensity at approximately average foliage height.

Natural daylight intensity on bright clear summer days in the greenhouses at Chicago commonly ranges up to 5000–6000 foot candles—occasionally even more. While these maximum values are much lower and are attained much less frequently than are the values of 10,000

foot candles or more in the natural distributional areas of the species under consideration (5), the relatively vigorous growth of all species (figs. 1–3) suggested that the lower light intensity at Chicago was not an important determining factor in the responses observed during the spring, summer, and early autumn months. It is probable that it is more favorable for growth in height than are the higher intensities (cf. BENEDICT, 5). In late autumn and winter at Chicago, however, with much lower intensities of natural light, most species became practically dormant, at least so far as growth and flowering were concerned. Some recent experiments with artificial supplemental light indicate that the low natural light intensity is the chief factor involved in this dormancy or limited growth in warm greenhouses in the winter months. For this reason, experiments on photoperiodic responses in these grasses have been conducted chiefly in the late spring and summer months.

Heating and ventilation of the greenhouses was so regulated as to minimize temperature differences among the various treatments and to provide favorable conditions for growth. The daily record from maximum-and-minimum thermometers placed on each truck and the growth observed indicate that the minor temperature differences were of little significance in affecting the photoperiodic responses. While the temperature values at Chicago were undoubtedly somewhat different from those in the native habitats, the photoperiodic responses are sufficiently fixed as not to be affected appreciably within the range of temperatures favorable for growth likely to be encountered by the species during their active periods, especially for the five strains from Arizona. Subsequent experiments have indicated that winter chilling is unnecessary to bring about reproductive activity in these strains. The results may probably be interpreted rather generally, therefore, although it is realized that photoperiodic responses are conditioned by numerous environmental factors (4, 6, 17, 20, 23), each of which might have different values in natural environments from those encountered in these experiments.



FIGS. 1–3.—Ten plants per pot 130 days after germination of (left to right) *Bouteloua gracilis*, *B. eriopoda*, *B. hirsuta*, *B. filiformis*, *B. rothrociki*, and *B. curtipendula:* fig. 1 (top), grown on 8-hour photoperiod; fig. 2 (middle), 12-hour photoperiod; fig. 3 (bottom), 16-hour photoperiod.

Favorable soil-moisture conditions were maintained at all times and nutrient levels in the soil were apparently adequate. Seedlings were thinned to ten per pot after the first month of growth, and while those remaining were selected somewhat for uniformity in size and spacing, the 100 plants of each species thus subjected to each light treatment through the summer probably represented a reasonably adequate and accurate sample of a population obtainable from the particular seed sources. In a few cases a slightly lower number than ten per pot was available because of insect injury in the early period of growth, and—in the case of *B. eriopoda*—because of uneven germination and growth in the first month (April), apparently due in part to a somewhat higher temperature requirement than in the other species. Because of the general similarity in competition within the various pots, the range in photoperiodic response among the 100 individuals (or less) of one species under similar light treatment must be ascribed more to genetic variation within the population than to lack of uniformity in the environment.

It became necessary, because of the luxuriant growth of the taller species, especially on 16-hour photoperiod, to provide supports of bamboo and twine for the tops. This inadequacy of the culms could not be ascribed to etiolation in response to low light intensity at Chicago, since they could easily have supported the foliage ordinarily produced by the various species, but rather it seemed to be due to the continuation of vegetative growth beyond the limit reached under natural environment.

Aside from general notes throughout the season, extensive data were collected, especially on flowering responses, during June when the plants were $2-2\frac{1}{2}$ months old. Late in August and early in September, when the plants were $4\frac{1}{2}-5$ months old, three pots (five for *B. eriopoda*) of each species from each truck were harvested by clipping the tops approximately 1 inch above the soil surface. Thus tops of approximately 60 plants (100 for *B. eriopoda*) of each species under each treatment were available for study. The roots and crowns were washed out of three of the harvested pots (four for *B. eriopoda*) of each species for each treatment at this time and saved for examination and dry-weight determination. The other three pots (six for *B. eriopoda*) of each species for each treatment were returned to the trucks and reciprocally transferred, so that for the ensuing period three pots of clipped plants of each species (six of *B. eriopoda*) were placed under each of the photoperiods, two of the three (four of the six of *B. eriopoda*) having previously grown under the other photoperiods. This transfer was made to see whether any "carry-over" effect of previous treatment remained in the crowns and roots of the clipped plants. Simultaneously, similar reciprocal transfers were made among the four unharvested pots of each species on similar treatment (except none for *B. eriopoda*), two pots of each species and treatment being left on the same treatment, while one was transferred to each of the other two conditions. In addition to notes during the ensuing growth period, data were obtained on these plants when the tops of those previously unclipped were harvested in early December, those of the remaining pots in middle February, 1942.

Most of the pots were then discarded, except those of *B. filiformis* and *B. curtipendula*, which were retained for experimentation the next summer (1942), but were placed on natural photoperiod in the ensuing interval until July 22,

1942. These latter plants resumed relatively vigorous growth in March, after being clipped in February, and many inflorescences were produced under natural photoperiod in late April and early May. For the remaining 2 months of long natural photoperiods until July 22, only vegetative tillers were produced. On that date all plants were clipped to within 1 inch of the ground, and a variable number of pots of each of the two species was placed under photoperiods varying by $\frac{1}{2}$-hour intervals from 13 to $15\frac{1}{2}$ hours, in an effort to determine more precisely the critical light period for these species in their second year of growth. For the six treatments, 9 hours of natural daylight were provided (8:00 A.M.–5:00 P.M.), supplemented by artificial illumination after 5:00 P.M. to provide total photoperiods of 13, $13\frac{1}{2}$, 14, $14\frac{1}{2}$, 15, and $15\frac{1}{2}$ hours. Supplemental illumination by Mazda lamps was given under frames covered with lightproof black cloth, at intensities of 50–100 foot candles, depending on plant height. Flowering responses were observed for the next 2 months. On September 21, the plants of *B. filiformis* were again clipped and data taken on the tops. The same was done with *B. curtipendula* on October 16. These pots were then returned to natural photoperiod.

Results

According to the recent definition of ALLARD and GARNER (3), and from the data in table 1 and figures 4 and 5, the five species with strains from Arizona, especially side-oats, slender, hairy, and Rothrock's grama, should probably be considered short-day plants, while blue grama from Montana falls—although not so precisely—into either long-day or indeterminate categories. The former failed to flower, or were delayed in flowering, on 16-hour photoperiod, in contrast with responses on 8- and 12-hour photoperiods; while the latter, although flowering, first on the shorter light periods, showed more rapid acceleration of flowering and eventually flowered more profusely on the longest photoperiod. In general, the five species represented by southern strains exhibited more rapid and vigorous flowering responses on 12-hour than on 8-hour photoperiod, suggesting better adaptation to the photoperiod nearest that under which they naturally flower in late July and August in Arizona, although not falling clearly into the intermediate group defined by ALLARD (1).

TIME REQUIRED FOR FIRST FLOWERING

The beginning and progress of flowering was measured by the emergence of recognizable inflorescences from the surrounding leaf sheaths. While this is a less accurate measure than are dissection methods (6, 9), which enable one to recognize spikelet initiation in the apical meristem, the time and materials available did not permit their use. Moreover, observations with these methods have indicated that development of inflorescences is very rapid in these forms and that initiation may occur after marked internodal elongation of the culm, in contrast with certain other grasses (9).

FLOWERING IN YOUNG PLANTS.—Table 1 shows the number of days after germination on which the first inflorescence appeared in each species on each treatment. The data here and elsewhere suggest a somewhat fundamentally different behavior of the two species, side-oats and slender, belonging to the subgenus Atheropogon, from that of the others. The lag in onset of even limited flowering on 16-hour photoperiod was greatest in them. All species flowered first on 12-hour photoperiod, or simultaneously on 8- and

12-hour light periods in this first season of growth from seed. All species flowered in less than 2 months after germination under some one of the treatments (Rothrock's grama in 30 days), in contrast with development in native environments, where this phase is often not reached until the second year or later.

CLIPPED RECIPROCAL TRANSFERS.—Comparable results were obtained after clipping and reciprocal transfers among treatments, in the autumn of 1941. Side-oats and slender grama then failed to flower on 16-hour photoperiod, irrespective of previous photoperiodic treatment, while they first showed inflorescences on the 27th and 18th day, respectively, after clipping when on 12-hour photoperiod, and on the 33rd and 18th day after clipping when on 8-hour photoperiod. There was a slight but not marked lag in the onset of flowering in those plants which, on 16-hour photoperiod prior to clipping, were then transferred to the shorter photoperiods. Any carry-over effect of previous treatment was very limited, however, and was not sufficient after clipping to result in flowering of plants which were transferred from the shorter to the longest photoperiod. Slender grama is obviously capable of very rapid response to the photoperiodic stimulus, a period of 18 days after clipping being sufficient for renewed growth with emergence of inflorescences. In the other three species with strains of southern origin, some flowering occurred on all treatments after clipping and transfer. All pots of hairy and Rothrock's grama flowered during the first month after clipping when on 8- or 12-hour photoperiods, but required 2–3 months on 16-hour photoperiod. Black grama did not grow well after clipping, many plants dying and others which had bloomed before clipping fail-

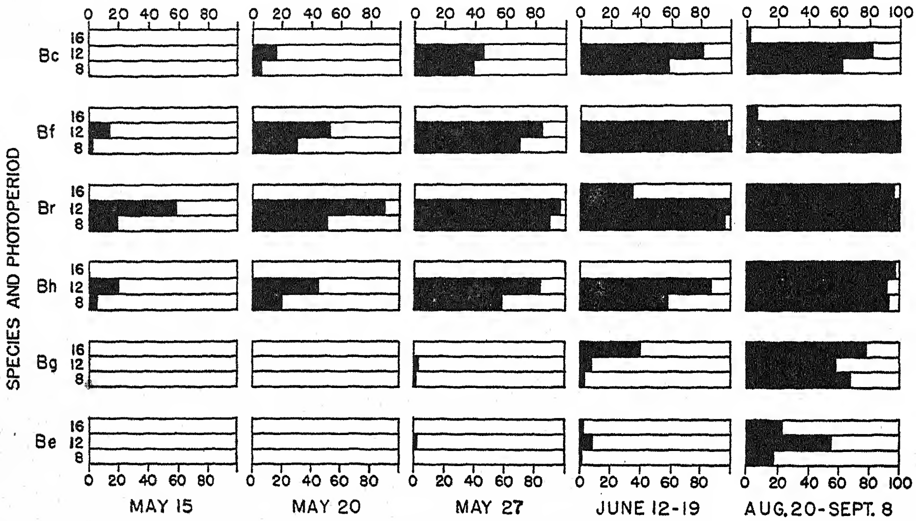


FIG. 4.—Percentages (shaded) of plants in flower (inflorescence emergent above surrounding leaf sheath) of six species of *Bouteloua*, subjected to photoperiods of 16, 12, and 8 hours per day after germination on April 6. Data for May based on 100 or more plants per species on each treatment; for June and August on 60 plants (100 for *B. eriopoda*) per species on each treatment. *Bc, B. curtipendula; Bf, B. filiformis; Br, B. rothrockii; Bh, B. hirsuta; Bg, B. gracilis; Be, B. eriopoda.*

ing to flower. Flowering did occur in the first 2 months on 8- or 12-hour photoperiods, but only in the third month on 16-hour photoperiod. Blue grama from Montana flowered only on 16-hour photoperiod after clipping, possibly confirming its long-day status, but also suggesting that too low light intensity, shown by BENEDICT (5) to be very unfavorable to this species in its flowering behavior, may also have been partly responsible. BENEDICT (4) had earlier classed blue grama as an indeterminate species and indicated that it also required high temperatures to flower, although he did not state the geographic source of his plants.

CRITICAL PHOTOPERIOD.—In the critical-photoperiod experiment on side-oats and slender grama in the summer of 1942, in which 2nd-year plants were distributed among six photoperiods after clipping to the ground, slender grama flowered in 24 days on 13-hour photoperiod, in 28 days on 13½ hours, and between the 28th and 38th days on 14 hours. It failed to flower in 61 days on 14½-, 15-, and 15½-hour photoperiods. Under similar treatment, side-oats grama flowered between the 41st and 61st days on 13½- and 14½-hour photoperiods but failed to flower on the others in 86 days. Under the conditions of this experiment, in which all plants received 9 hours of natural daylight daily, the upper critical photoperiod for these two species lies between 14 and 14½ hours. This is approximately the maximum photoperiod to which they are subjected during active growth in southern Arizona. Failure of side-oats grama to flower on 13-hour photoperiod suggests that this strain, under certain experimental conditions, would fall into ALLARD'S (1) intermediate class, although the other experiments do not bear this out, since the same plants flowered on 8-hour photoperiod in their first year of growth. This indicates the need for caution in interpreting the results of single experiments on limited material and suggests that the range of photoperiods optimum or even favorable for flowering in this species may be much narrower under somewhat adverse conditions, such as the limited duration of high light intensity (9 hours for all treatments) in this experiment, than under more favorable conditions. This is borne out by its failure to flower again on natural photoperiod, although resuming vegetative growth after clipping in October, 1942, until both photoperiod and light intensity apparently became favorable in April, 1943. Slender grama plants, grown under 13, 13½, and 14 hours in the critical-photoperiod experiment, flowered again in 25, 22, and 22 days, respectively, after clipping in late September when placed on natural photoperiod. Plants previously on longer photoperiods (14½, 15, 15½ hours) did not flower for over 40 days after clipping, suggesting a more pronounced carry-over effect of previous treatment than in the clipping experiments in 1941. All slender grama plants

TABLE 1

NUMBER OF DAYS UNTIL FIRST FLOWERING,* AFTER GERMINATION ON APRIL 6, 1941, OF SIX SPECIES OF BOUTELOUA ON PHOTOPERIODS OF 8, 12, AND 16 HOURS PER DAY

SPECIES	PHOTOPERIOD		
	8 hours	12 hours	16 hours
B. curtipendula	44	42	114
B. filiformis	39	39	106
B. rothrockii	30	30	57
B. hirsuta	39	36	80
B. gracilis	51	51	63
B. eriopoda	>51<74	51	>51<74

* Measured by emergence of inflorescence from surrounding leaf sheath.

continued to flower on natural photoperiod through autumn, winter, and spring months, until the longer photoperiods of late spring became unfavorable, after which they grew vegetatively. Side-oats definitely requires higher light intensities for both satisfactory growth and flowering than does slender grama.

Rate of flowering

Experiment, Summer 1941.—The previous discussion is based primarily on the length of time required for the first plant to flower in each species and treatment. Results statistically more significant are based on the sequence of flowering of all plants in each population (fig. 4). Data are presented of the percentage of plants which were flowering or had flowered (at least one inflorescence emerging or emergent per plant) on five different dates, ending with the harvest in late August and September. They confirm the previous conclusions, that these strains of all species (except blue grama) should probably be regarded as short-day plants, either because a very low percentage flowered on 16-hour photoperiod or because plants which ultimately flowered were delayed on 16-hour photoperiod as compared with the other treatments. Side-oats, slender, Rothrock's, and hairy grama attained nearly their final percentages of plants flowering on the short photoperiods before any flowering occurred on the 16-hour photoperiod, even though in the latter two species practically all plants flowered, while in the first two species flowering was limited to very few plants on 16-hour treatment. Black grama did not follow this general pattern, the plants on 12-hour photoperiod continuously showing the highest percentage in flower, so that at harvest more than twice as many of them had flowered as in the other treatments which showed similar and low values. In blue grama, which flowered first on 12- and 8-hour photoperiods, the percentage of plants in flower on 16-hour photoperiod rapidly increased (data for May 27 and June 12–19), and ultimately this latter treatment had the highest value, although the final data suggest an indeterminate effect. All the data also indicate considerable variation in all species on each treatment as to time of first initiation of inflorescences in individual plants. This was more marked on the shorter than on the 16-hour photoperiod. In fact, at harvest all types of morphological variation were less marked in the latter treatment, especially in side-oats, slender, Rothrock's, and hairy grama. The longer period of strictly vegetative growth for the plants on 16-hour photoperiod permitted more uniform growth, contrasting with the greater difference in time of floral initiation by the various individuals on the shorter photoperiods, which tended to accentuate vegetative differences within each species.

Clipped reciprocal transfers.—Rate of flowering in the clipped reciprocal transfer experiment followed the same general tendencies shown in figure 4, except that side-oats and slender grama failed to flower on 16-hour photoperiod, while blue grama did not flower on 8- and 12-hour photoperiods. Percentages of plants which ultimately flowered in other species and treatments were mostly lower than those shown in figure 4, the percentages for Rothrock's and hairy grama on 16-hour photoperiod proportionately much more so than on 8- and 12-hour treatments. Delay in initiation on the 16-hour photoperiod caused the flowering period to coincide with the season of unfavorable light intensity and limited growth in general, which finally termi-

nated the flowering responses of all species except slender grama.

Unclipped reciprocal transfers.—Most obvious responses with these transfers were obtained with side-oats and slender grama previously grown on 16-hour photoperiods and transferred to the shorter light periods. In slender grama, all plants—few of which had flowered earlier—initiated inflorescences within 3 weeks after the transfer, either at the tips of elongated vegetative culms and branches from the upper nodes of the latter, or from tillers which were unelongated previous to transfer. In side-oats grama similar results were obtained, although the time required was longer (4–6 weeks) and the percentages of plants originally nonflowering which ultimately flowered were smaller. In this species the inflorescences more generally appeared on new basal tillers. After the reciprocal unclipped transfer of these two species from shorter to long photoperiod, the appearance of new inflorescences ceased in about 2 weeks. In slender grama, vegetative branches from old flowering culms then developed from buds on their upper nodes, these branches overtopping the tips of the old inflorescences. Similar vegetative branches developed in side-oats grama, although the elongation of basal, previously unelongated tillers was more common. The latter occurred in slender grama also. In the similar transfers of Rothrock's and hairy grama from short to long photoperiod there was a delay in inflorescence initiation on subsequently formed tillers, while in the reciprocal transfer this initiation was accelerated. The results on blue grama cannot be summarized, and there were no unclipped transfers of black grama. Unclipped plants of all species left on previous treatment when others were transferred showed a continuation of the pattern already indicated in figure 4 for their earlier growing period. All continued to flower or failed to flower, as at the time of harvest of the other pots, and served to verify the interpretations of the effect of transfers already given.

Growth in height

As is generally true in short-day plants, maximum heights were positively correlated with length of photoperiods to which the plants were subjected. Maximum height of a clipped plant was measured from the cut stem ends to the tip of the longest outstretched leaf or to the tip of the inflorescence, whichever gave the greater value. Although the values given are therefore slightly higher than the average standing heights of the plants, they are in proportionate agreement (figs. 1–3, 5). The most striking features of this response were the much greater heights attained by all species on 16-hour photoperiod, whether flowering or nonflowering, than they reach under natural conditions in the field. In figure 5, mean values are given for all plants (solid bars) and for those plants which flowered (solid bars plus hatching) in each species on each treatment. Similar proportionate values were measured earlier in the growing season, differences being observable in the amount of elongation of the first and second foliage leaves in the first week of growth. Length and width of leaf blades and sheaths, length of internodes, and diameter of stems and roots were much greater in the plants on 16-hour photoperiod than in the others. While the dry-weight yield (fig. 6) was therefore much greater in the plants on long photoperiod in four of the species, the forage would have been much less palatable. No effort was made to correlate these responses

with internal anatomical conditions, as STUCKEY (26) has recently done with orchard grass.

Unclipped plants of slender and side-oats grama, left on 16-hour photoperiod after most plants were clipped in August, continued to grow vegetatively and to increase in length, reaching average heights of 130 and 176 cm. by mid-December, when they were harvested. Unclipped plants, previously on 16-hour photoperiod, grew only slightly in length after transfer to shorter light periods, the increase being mainly due to elongation of inflorescences which initiated. Simultaneous transfers of unclipped slender and side-oats grama from 8- and 12-hour to 16-hour treatments resulted in average height gains of 20–40 cm. over their values in August, in contrast with little increase in plants allowed to remain on short photoperiods.

Height growth in the clipped reciprocal transfers confirmed the results of the earlier experiment, all species being tallest on 16-hour photoperiod at the time of harvest in February, 1942. Heights were much shorter than in the August harvest, however, especially in black grama, probably because of the less favorable light intensity. There was little obvious effect of previous photoperiodic treatment on the height attained in the various pots of each species on each photoperiod after clipping and transfer, except in slender and side-oats grama. Plants of these species remaining on 16-hour treatment were taller than those transferred to it from shorter photoperiods.

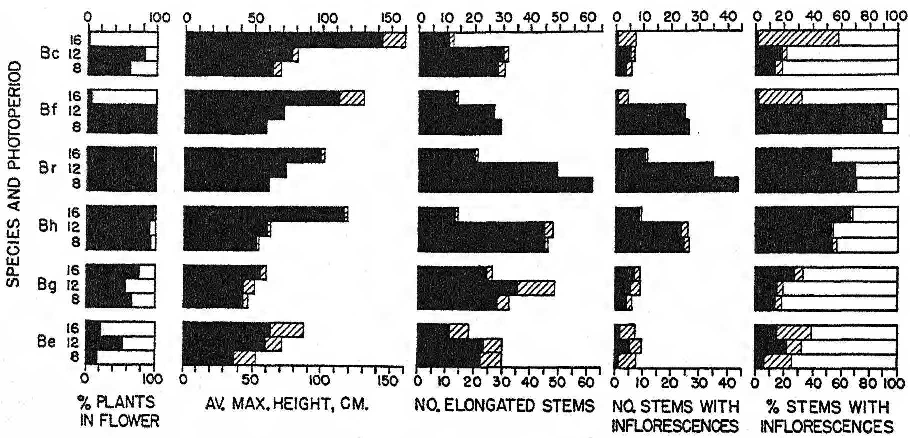


FIG. 5.—Growth and flowering of six species of *Bouteloua*, subjected to photoperiods of 16, 12, and 8 hours per day after germination, and harvested when $4\frac{1}{2}$–5 months old. Solid black bars are averages of 60 plants (100 for *B. eriopoda*) for each species on each treatment; solid black bars plus hatching are averages of all plants which flowered on each treatment: *Bc, B. curtipendula; Bf, B. filiformis; Br, B. rothrockii; Bh, B. hirsuta; Bg, B. gracilis; Be, B. eriopoda.*

TILLER DEVELOPMENT AND CULM ELONGATION

Internodal elongation of primary axis and individual tillers apparently began sooner in all species on the shorter photoperiods than on the 16-hour light period. Their growth was also terminated much sooner, either as a consequence of obvious development of inflorescences, or without

it, than on the long photoperiod. Although internodal elongation is sometimes taken as the first easily observable evidence of reproductive activity in grasses, it need not be accompanied or soon followed by initiation of spikelet primordia. Recent observations on side-oats grama have shown that vegetative development of such axes may continue from the apical meristem for more than elongation. The August data are averaged on a plant basis for all plants, and for flowering plants only (fig. 5). Fewer tillers with elongated internodes were produced by the plants on 16-hour photoperiod in all species than on the other treatments. The differences in such numbers between the 8- and 12-hour treatments are not statistically significant, except in Rothrock's and blue grama, the

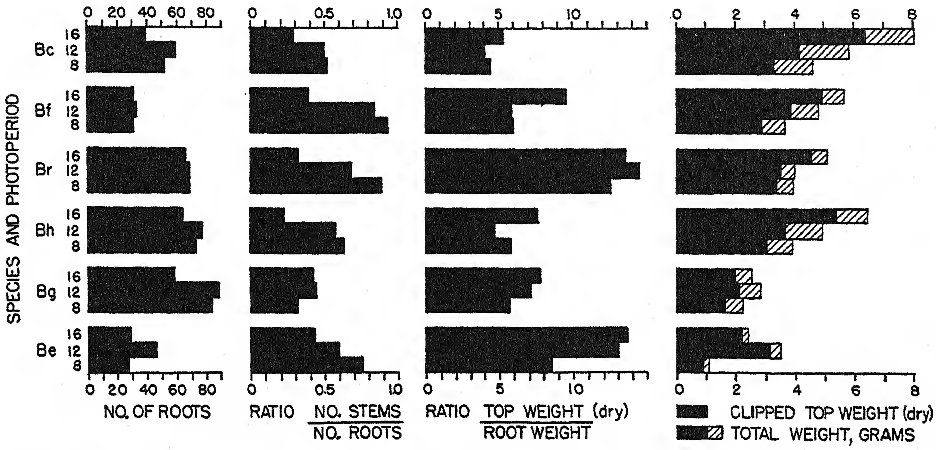


FIG. 6.—Growth of six species of *Bouteloua*, subjected to photoperiods of 16, 12, and 8 hours per day after germination and harvested when 4½–5 months old. Averages of 30 plants per species on each treatment. *Bc, Bouteloua curtipendula; Bf, B. filiformis; Br, B. rothrockii; Bh, B. hirsuta; Bg, B. gracilis; Be, B. eriopoda.*

a year without any suggestion of development of inflorescences, these axes reaching lengths of more than 2 meters.

In the 1941 experiments no data were obtained on the total numbers of tillers formed in the various species and treatments, although they were much the lowest in plants on 16-hour photoperiod. Tillers with one or more elongated internodes were counted on all plants harvested in August, however, and also on previously unclipped slender and side-oats grama harvested in December. These were counted by stripping away the surrounding leaf sheaths to verify the former showing the greatest number on 8-hour photoperiod, the latter on 12. The cessation of height growth of individual axes on the shorter photoperiods resulted in continued production of basal tillers, while the apical meristems remained active and dominant for longer periods on 16-hour photoperiod, so that the rate of basal tiller initiation and elongation was much slower.

New tillers developed throughout the season on all species and treatments. For example, the number of elongated culms doubled in slender and side-oats grama from August to December, 1941, on all

treatments in those plants which were neither clipped nor transferred to a different photoperiod. Tiller counts on clipped plants in February were of little value, since differential death of plants or parts of their crowns after the August clipping confuses the results, but the same tendencies are suggested.

Development of inflorescences

The numbers of stems per plant arising from the crown and on which one or more inflorescences had been developed by August and September are shown in figure 5, on both a total-plant and flowering-plant basis, together with the total percentage of elongated stems which had formed inflorescences. Both sets of data indicate a much more profuse flowering tendency of slender grama on 8- and 12-hour photoperiods, and of Rothrock's and hairy grama on all photoperiods, at the time of the August harvest than in the other three species, when measured by the number of stems involved. Similar results were obtained in the other experiments. The data are not absolute values for the total numbers of inflorescences produced. Individual tillers in the cases of side-oats, Rothrock's, black, and especially slender grama, branched to give rise to more than one inflorescence (up to fourteen on a single tiller in slender grama). This condition was not found in hairy or blue grama. In general, the total number of stems bearing inflorescences was largest in all species on one of the shorter photoperiods. Blue grama is an exception, in that the value for plants on 16 hours of light per day approximately equaled that for those on 12 hours. When based on all plants, the percentages of stems flowering per plant were greater on the short photoperiods for side-oats, slender, Rothrock's, and black grama, whereas they were higher on 16-hour photoperiod for hairy and blue grama. When based on flowering plants only, the flowering response seems most vigorous by this measurement on the 16-hour photoperiod for all species except slender and Rothrock's grama. However, the numbers of side-oats and slender grama plants which flowered on 16-hour photoperiod, and those of black grama which flowered on 8- and 16-hour photoperiods, were so few that probably little significance should be attached to the results. They suggest that when an individual plant is able to flower on all photoperiods, the 16-hour light period may be more favorable for profuseness of flowering than the others.

A similar conclusion is made with more validity on comparing the length of inflorescence, the number of spikes per inflorescence, the length of spike and number of spikelets per spike, and the percentage of sterile florets and spikelets, among all the plants which flowered on all treatments. In all species, observations on these criteria, especially in June and August, indicated a somewhat greater favorability of the 16-hour photoperiod for vigorous flowering. This conclusion need not negate the general short-day status of all species except blue grama. It suggests, however, that conditions of the experiment were not very favorable for continued growth of inflorescences which were more rapidly initiated on the shorter photoperiods. The limitation of 8 hours of natural daylight on both 8- and 12-hour photoperiods, in contrast with the full natural photoperiod available to plants on 16 hours of light, may account in large measure for this response, as well as for less vigorous vegetative growth. In nature the five species with strains from Arizona mature their inflorescences on a decreasing photoperiod of high light in-

tensity in July and August, although it is still in excess of 8 or 12 hours when they are mature.

The contrast in vigor of spikes between the 8- and 16-hour treatments was most marked in hairy grama. On 16-hour photoperiod, five spikes per inflorescence were sometimes produced, ranging in length up to 2 inches and bearing spikelets almost to the tip of the rachis, so that the sterile rachis tip often taken as diagnostic of this species (12) was practically obscured. In contrast, on 8-hour photoperiod, inflorescences occasionally consisted of only one poorly developed spike having few spikelets and a rachis extending $\frac{1}{2}$ inch or more beyond them. In extreme cases no spikelets were developed, a spike consisting of a sterile rachis $\frac{1}{4}$–$\frac{1}{2}$ inch long. There was great variation among individual plants in this regard.

Viable caryopses were apparently produced in these experiments only by slender grama, which did so on all treatments. Its seedlings often appeared in the pots in late summer and autumn. It is not known whether the failure was due to lack of pollination in these cross-pollinated species under greenhouse conditions, or whether physiological causes were involved.

Number of roots

Plants of each species for each treatment were washed out of the pots in August and the numbers of crown roots counted (fig. 6). The numbers were higher in five of the species on the shorter than on the 16-hour photoperiod, although not significantly so except in side-oats and blue grama. In black grama the numbers of roots on plants on 8- and 16-hour photoperiods were approximately equal. The value of the ratio, number of elongated stems/number of roots, was inversely correlated with length of photoperiod for all species except blue grama (fig. 6). This is owing chiefly to the strong inverse correlation between number of stems and length of photoperiod. These results indicate that the greatest numbers of root primordia were initiated per tiller under long photoperiods, a condition probably correlated in turn with the greater number of basal nodes and internodes per tiller which matured before these tillers elongated.

Top and root weights

Dry weights of tops (less crowns) were determined for all plants after clipping and showed tendencies in all experiments comparable with those in figure 6, where data are given for weights of clipped tops (solid bars) and for the combined weights of clipped tops, crowns, and roots (solid bars plus hatching). There were positive correlations between dry weights and length of photoperiod, except in blue and black grama. This is a result generally found in short-day plants. Average top weights of slender and side-oats grama plants harvested in December, previously unclipped and remaining on the same photoperiod since germination in April, were approximately double those of the same species and under similar treatment but harvested in August.

Top-weight/root-weight ratios showed no consistent tendency (fig. 6) among treatments but emphasized the high proportion of top weight (clipped top weight plus crowns) in the total weight, a result which would not be expected if these plants were grown in native environments.

Discussion

The results show definitely that certain strains of some species of *Bouteloua* are

sensitive to photoperiod. Within the limits of the experiments, side-oats and slender grama were most so. These species belong to a different subgenus (Atheropogon) from the others. Slender grama is a "typical" short-day plant in all its responses, practically all individuals failing to flower on photoperiods above the critical one, which lies between 14 and $14\frac{1}{2}$ hours. They flowered profusely on photoperiods down to 8 hours, under variable conditions of light intensity. This is in accord with the probable evolutionary history of the species. Its present distribution indicates origin in low latitudes, since it now occurs only in latitudes below 35°, where maximum photoperiods do not exceed its apparent critical one. Northward extension of the strain may well be limited in part by this adaptation to short photoperiods. Other species in the same subgenus, except side-oats grama, are found only in low latitudes.

Side-oats grama ranges to higher latitudes in both South and North America (to 50° N. for the latter). The results here reported show that the strain from Arizona consists chiefly of short-day plants with a critical photoperiod between 14 and $14\frac{1}{2}$ hours. It should be remembered, however, that a few plants of both side-oats and slender grama were able to flower, although delayed in so doing, on 16-hour photoperiod. These individuals, especially side-oats grama, may be of considerable significance from the standpoint of varietal adaptation, even though of little importance in adjustment to their Arizona environment. Additional studies (19), not yet reported in full, show that investigated strains of side-oats grama from northern United States consist chiefly of individuals which may be classed as long-day plants, while those from southern United States are mostly short-day (or possibly intermediate). Since the species has undoubtedly spread from south to north in the United States, these results suggest that the northern populations of predominantly long-day plants may have been evolved during this extension of range from populations which were primarily short-day in response in the lower latitudes. If so, a thorough genetic analysis (with respect to photoperiodic response) of a population such as that from southern Arizona, which includes a few individuals not showing the "typical" response, would be of interest. It might throw light upon the possible evolutionary mechanism which led to the present nice adjustment of response to the seasonal range in length of photoperiod as the area was extended from south to north. For example, it seems probable that some of the individuals showing less typical photoperiodic responses, such as those from Arizona able to flower on 16-hour photoperiod, would have been the ones perpetuated through natural selection in the northward migration; although no answer is suggested as to why the northern strains are now chiefly long-day rather than indeterminate. This variability in response to photoperiod in local populations should be taken into consideration in selection and breeding programs, either in interpreting "earliness" in flowering of certain individuals or in cross-breeding of desirable strains from different latitudes.

The investigated members of the subgenus Chondrosium (Rothrock's, hairy, blue, and black grama), probably also originating in the low latitudes, to which many (including black and Rothrock's) are still restricted, apparently are less sensitive to photoperiod as far as flower-

ing responses are concerned. They do exhibit many vegetative responses typical of short-day plants. The strains of the three species from Arizona (black, Rothrock's, and hairy grama) fell into the less typical group of short-day plants (3) which are delayed in flowering on long photoperiods as compared with short ones. If they have a well-defined critical photoperiod, it is longer than any provided in these experiments. Photoperiodic conditions and adaptations have probably been of less importance in the evolutionary development of these species. The possible delay in initiation of reproductive activity in these strains, as well as a midsummer inhibition of flowering in slender and side-oats grama when they all renew vegetative growth under the long photoperiods of the well-defined summer rainy season in southwestern United States, probably leads to greater forage production than if they were completely indeterminate and were to initiate flowering earlier in the season.

The blue grama strain from Montana most closely approaches the indeterminate category, although it showed long-day tendencies. These are in accord with the photoperiodic conditions encountered during the Montana growing season, although the species probably originated in lower latitudes. LAVIN's studies (15) suggest that in its spread to the north it has become adapted to the increasing range of photoperiods encountered in a somewhat different fashion from that of side-oats grama.

The results indicate that length of photoperiod is undoubtedly a factor of considerable importance in affecting the type of vegetative growth produced at different seasons of the year by all the species investigated; they suggest strongly that knowledge of such responses to photoperiod, as well as flowering responses, would be helpful in guiding efforts to obtain desirable strains of these species through selection and breeding, either for forage production or for use in reseeding overgrazed or denuded areas. Such knowledge certainly would aid in interpreting the variations in growth habit which have been described (10, 14, 21, 24) when strains from different latitudes are grown side by side.

Summary

1. Lots of 100 plants each of side-oats grama (*Bouteloua curtipendula*), slender grama (*B. filiformis*), Rothrock's grama (*B. rothrockii*), hairy grama (*B. hirsuta*), black grama (*B. eriopoda*), and blue grama (*B. gracilis*) were grown continuously for $4\frac{1}{2}$–5 months after germination on April 6 on photoperiods of 16, 12, and 8 hours in the greenhouse. Reciprocal transfers were then made among treatments, both with and without simultaneous clipping of the tops, and the plants were allowed to grow for another 4–6 months. Southern Arizona, except for blue grama from Montana, was the seed source of all species.

2. Growth and flowering responses showed that these strains of slender and side-oats grama, which belong to the same subgenus (Atheropogon), are "typical" short-day plants, most individuals failing to flower on a 16-hour photoperiod. A critical-photoperiod experiment showed this value to lie between 14 and $14\frac{1}{2}$ hours.

3. The other four species, belonging to the subgenus Chondrosium, made less decisive flowering responses. The species with strains from Arizona were considerably delayed in flowering on the longest photoperiod, and these strains

should probably be regarded as short-day plants. Blue grama from Montana was more or less indeterminate, with some long-day tendencies in flowering behavior.

4. The five species with strains from Arizona exhibited more or less typical short-day vegetative behavior. The total numbers of tillers, of tillers bearing inflorescences, and of crown roots were inversely correlated with length of photoperiod to a greater or less degree in most species; while average maximum height, average dry weights of roots and tops, and vigor of individual inflorescences were correlated positively with length of photoperiod.

5. The genus is definitely sensitive to photoperiod, and its effect should be considered in interpreting growth habits of the species in nature and in selection and breeding programs designed to develop strains more desirable for forage production or for use in artificial revegetation of overgrazed or denuded areas.

It is a pleasure to acknowledge with appreciation the assistance of N. J. SCULLY, JOHN DIBBERN, and FRED LAVIN in the experimentation.

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LITERATURE CITED

1. ALLARD, H. A., Complete or partial inhibition of flowering in certain plants when days are too short or too long. Jour. Agr. Res. 57:775–789. 1938.
2. ALLARD, H. A., and EVANS, M. W., Growth and flowering of some tame and wild grasses in response to different photoperiods. Jour. Agr. Res. 62:193–228. 1941.
3. ALLARD, H. A., and GARNER, W. W., Further observations on the response of various species of plants to length of day. U.S.D.A. Tech. Bull. 727. 1940.
4. BENEDICT, H. M., Effect of day length and temperature on the flowering and growth of four species of grasses. Jour. Agr. Res. 61:661–672. 1940.
5. ———, Growth of some range grasses in reduced light intensities at Cheyenne, Wyoming. BOT. GAZ. 102:582–589. 1941.
6. BORTHWICK, H. A., PARKER, M. W., and HEINZE, P. H., Effect of photoperiod and temperature on development of barley. BOT. GAZ. 103:326–341. 1941.
7. EVANS, M. W., Relation of latitude to certain phases of the growth of timothy. Amer. Jour. Bot. 26:212–218. 1939.
8. EVANS, M. W., and ALLARD, H. A., Relation of length of day to growth of timothy. Jour. Agr. Res. 48:571–586. 1934.
9. EVANS, M. W., and GROVER, F. O., Developmental morphology of the growing point of the shoot and the inflorescence in grasses. Jour. Agr. Res. 61:481–520. 1940.
10. FULTS, JESS L., Somatic chromosome complements in *Bouteloua*. Amer. Jour. Bot. 29:45–55. 1942.
11. GRIFFITHS, DAVID, The grama grasses: *Bouteloua* and related genera. Contrib. U.S. Nat. Herb. 14:343–444. 1912.
12. HITCHCOCK, A. S., Manual of the grasses of the United States. U.S.D.A. Misc. Publ. 200. 1935.
13. HOOVER, M. M., Native and adapted grasses for conservation of soil and moisture in the Great Plains and Western States. U.S.D.A. Farmers Bull. 1812. 1939.
14. HOPKINS, HAROLD, Variations in the growth of side-oats grama grass at Hays, Kansas, from seed produced in various parts of the Great Plains region. Trans. Kansas Acad. Sci. 44. 1941.
15. LAVIN, FRED, Variations in the responses of different geographic strains of blue grama grass to photoperiod. Unpublished thesis, University of Chicago. 1943.
16. LIGHTLE, T., Unpublished thesis, University of Arizona. 1939.
17. MCKINNEY, H. H., and SANDO, W. J., Earliness of sexual reproduction in wheat as influenced by temperature and light in relation to growth phases. Jour. Agr. Res. 51:621–641. 1935.
18. OLMSTED, C. E., Growth and development in range grasses. I. Early development of *Bouteloua curtipendula* in relation to water supply. BOT. GAZ. 102:499–519. 1941.
19. ———, Photoperiodic responses in twelve geographic strains of side-oats grama, *Bouteloua curtipendula*. (Abstract.) Bull. Ecol. Soc. Amer. 23:72–73. 1942.

20. PARKER, M. W., and BORTHWICK, H. A., Effect of variation in temperature during photoperiodic induction upon initiation of flower primordia in Biloxi soybean. BOT. GAZ. **101**: 145–167. 1939.

21. RIEGEL, ANDREW, A study of the variations in the growth of blue grama grass from seed produced in various sections of the Great Plains region. Trans. Kansas Acad. Sci. **43**:155–171. 1940.

22. ———, Life history and habits of blue grama. Trans. Kansas Acad. Sci. 44. 1941.

23. ROBERTS, R. H., and STRUCKMEYER, B. ESTHER, The effects of temperature and other environmental factors upon the photoperiodic responses of some of the higher plants. Jour. Agr. Res. **56**:633–678. 1938.

24. SAVAGE, D. A., Grass culture and range improvement in the central and southern Great Plains. U.S.D.A. Circ. 491. 1939.

25. SHEPHERD, H. R., Studies in breaking the rest period of grass plants by treatments with potassium thiocyanate and in stimulating growth with artificial light. Trans. Kansas Acad. Sci. **41**:139–153. 1938.

26. STUCKEY, IRENE H., Some effects of photoperiod on leaf growth. Amer. Jour. Bot. **29**:92–97. 1942.

27. UNITED STATES FOREST SERVICE: Range plant handbook. Washington. 1937.

EFFECTS OF GROWTH-REGULATING SUBSTANCES ON PROPAGATION OF GOLDENROD

C. L. HAMNER[1] AND P. C. MARTH[2]

Material and methods

Solidago leavenworthii, one of the plants whose rubber-producing potentialities are being extensively investigated at the present time, is available in a considerable number of selected clones propagated mainly from cuttings. Various clones differ considerably in the rubber-producing capacities of their leaves, resistance to disease, degree to which they lose their lower leaves in late season, and other characters that affect the yield of rubber.

Although growth substances have been found effective in the rooting of cuttings of many kinds of plants, apparently they have not been tested with goldenrod, for no reference to their use with this plant is found in a recent review of the subject (2).

The material for this investigation was obtained through the Rubber Investigation Project from plantings at Savannah, Georgia. It consisted of plants from four clones—3 S-4, 3 S-40, 3 S-79, and 3 S-91—that had been selected for their high yield of rubber. The plants had been started from cuttings planted in Savannah on March 30, 1942, in Blanton loamy fine sand. In December the plants were dug and divided into stolons and stems and shipped the same day to Beltsville, Maryland, where the material was prepared for treatment. The stolons were firm and succulent, but the stems were old and woody, had lost their leaves, and become brown in patches. These old stems were used to compare their rooting abilities and to study the effects of growth regulators on such rooting. Other shipments of stolons were received until late in March, and cuttings of stolons were made from December until April.

Cuttings from young stems were later obtained from plants grown in the greenhouse from the stolon cuttings. This material was available about April 1, 1943, and cuttings of it were made from that time until June, 1943.

The growth regulators used were indolebutyric acid, α-naphthalene acetamide, β-naphthoxyacetic acid, α-naphthaleneacetic acid, and a mixture of equal parts of them. The carriers for these substances were either powdered talc or water.

The concentrations of the growth regulators in the powder were 50, 200, and 1000 p.p.m. In making the talc dust, a known amount of the growth regulator was first dissolved in a few cubic centimeters of 95% ethyl alcohol and added to a measured quantity of talc to form a paste. This mixture was dried in an electric oven at about 60°–70° C. and then reduced to a fine powder (2). The lower one-third of the cuttings was dipped in the dust and any excess shaken off. In water the concentrations were 10, 25, 50, and 100 p.p.m., secured by adding a solution of the growth substance in 95% alcohol to the required amount of water. The amount of alcohol used was such that it was less than 0.1% in the final solution. The lower one-third of each cutting was soaked in the solution for 3 or 18 hours.

The stem cuttings were 6 and 3 inches long and the stolons 2 inches and ½ inch long. Stem cuttings were further classified according to diameter as large, medium, and small and according to position on the stem as base, middle, and tip. The stems from which cuttings were made were also classified according to

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their condition and apparent age into young, mature, and over-mature. The over-mature stems had lost their leaves and some of the outer tissues were apparently beginning to die. Stems of both of the other lots were still leafy; those designated as young were 1–2 feet tall and the mature ones 2–5 feet. In most cases forty cuttings were used for each treatment.

After treatment, the cuttings were placed upright in the substrate, basal end down, with about two-thirds below the surface. They were planted at about 1-inch intervals in rows 4 inches apart. In one experiment in which $\frac{1}{2}$-inch stolon cuttings were used, the cuttings were placed horizontally $\frac{1}{2}$ inch below the surface.

Two kinds of substrate were used. One was a sandy, river-bottom type of soil and the other a potting mixture of sand, muck, and soil. The pH of the former was 5.6 and of the latter, 6.7. Between January and April, temperatures of the substrate ranged 75°–85° F. and during May and June, 80°–95° F. The greenhouse was given a light coat of whitewash on the glass about May 15, reducing the light intensity about 25%. When sand was used it was saturated with water at the start of the experiment but received no further watering until after rooting had begun. When soil was used, it was given a thorough soaking at the start of the experiment and light sprinklings every day. The soil, except immediately after sprinkling, was drier than the sand.

Results

Stolons

In January, 1942, stolon cuttings were grown both in sand and in soil to determine whether both types of media would be suitable for use in later experiments. Both media proved satisfactory, but if cuttings were allowed to become established (that is, if the root systems were allowed to develop), the roots grew faster and more extensively in the soil. Ten days (in most cases) after they were first placed in the media, the cuttings were examined to determine any differences in time or amount of rooting resulting from applications of growth substances. If the cuttings were left in the media longer, substrate nutrition, aeration, and other factors might have tended to mask the effects of the growth regulators upon rooting. Stolon cuttings produced the greatest number and amount of roots when the substrate temperature was 70°–80° F., in contrast to lower temperatures. The 3-inch cuttings placed upright in the substrate produced more roots and tops than did the $\frac{1}{2}$-inch ones placed horizontally below the soil surface. Of the latter, more than 50% failed to grow, and the remainder did not grow vigorously (fig. 1). Rotting was much more pronounced in the small underground pieces.

It was apparent from preliminary trials that water was not as satisfactory a carrier for the growth substance as talc dust in the rooting of stolon cuttings. As a result it was used in only a few trials. Indolebutyric acid, and a mixture of indolebutyric, naphthaleneacetic, naphthoxyacetic acids, and naphthalene acetamide were used at 10 and 50 p.p.m. Top growth of cuttings soaked for 18 hours in an indolebutyric-acid solution at 50 p.p.m., or in a mixture at 50 p.p.m., was inhibited. Soaking in indolebutyric acid at 10 p.p.m. for 18 hours produced slightly more roots than controls which had been soaked 18 hours in water only (fig. 2). Soaking in an indolebutyric-acid solution at 50 p.p.m. for 3 hours produced many more roots than the controls,

whether the substrate was at 60°, 70°, or 80° F. Cuttings soaked in indolebutyric acid at 100 p.p.m., or merely dipped at 1000 p.p.m., showed marked top inhibition (fig. 3).

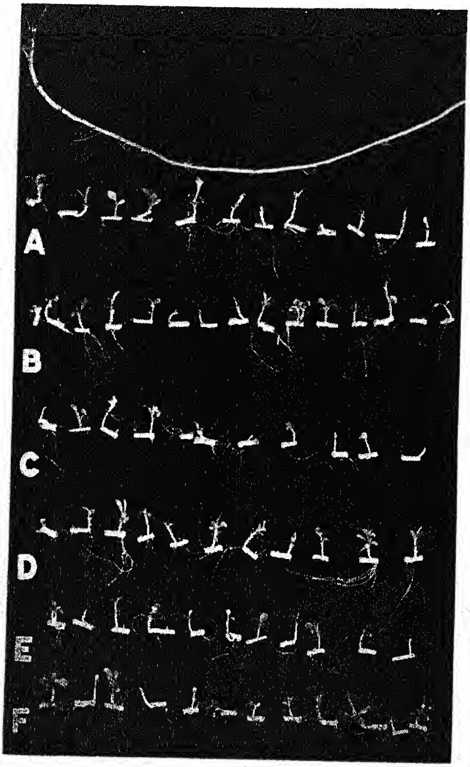

FIG. 1.—Effect of growth substance in talc on ½-inch stolon cuttings of goldenrod. *A*, untreated; *B*, talc control; *C*, ibc, 1000 p.p.m.; *D*, ibc, 200 p.p.m.; *E*, ibc, 50 p.p.m.; *F*, mixture of ibc, nac, nad, noac, total of 200 p.p.m. At top, representative stolon before division.

Stolon cuttings dipped in growth substance dispersed in talc produced more numerous and vigorous roots than did those without treatment. Application of all the growth substances except naphthoxyacetic acid resulted in increased rooting, at least at certain concentrations. Cuttings dipped in dusts containing indolebutyric acid developed the most vigorous root system, as to both number and volume. Indolebutyric acid was most effective at 200 and 1000 p.p.m. (fig. 4, table 1). Roots from cuttings

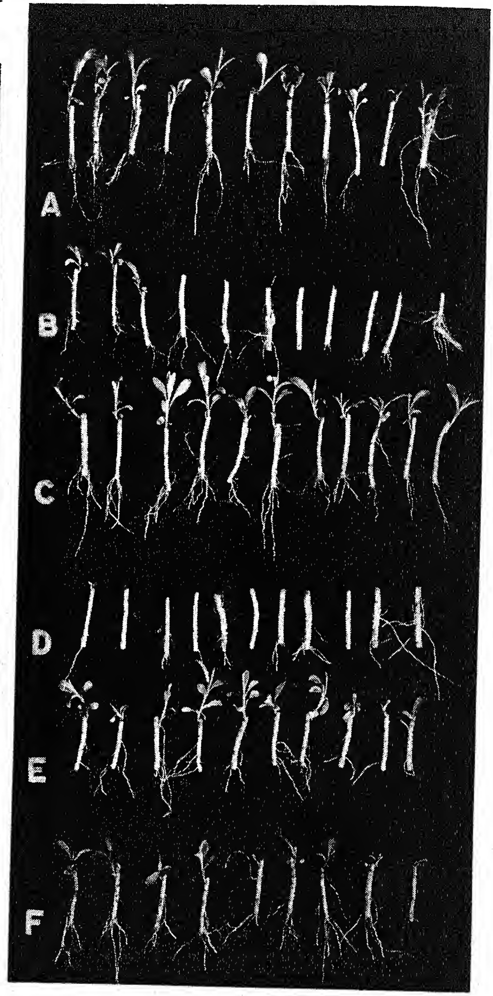

FIG. 2.—Stolon cuttings of goldenrod soaked in solutions of growth regulators for 18 hours. *A*, controls, water only; *B*, ibc, 50 p.p.m.; *C*, ibc, 10 p.p.m.; *D*, mixture of ibc, nac, nad, and noac, total of 50 p.p.m.; *E*, mixture as in *D*, total of 10 p.p.m.; *F*, nac, 10 p.p.m.

treated with indolebutyric were very slender and long in contrast to the shorter and more sturdy roots of the controls.

Cuttings treated with naphthalene-acetic acid at 200 p.p.m. developed a

root system comparable in appearance with those from treatment with indolebutyric at 200 and 1000 p.p.m. Not as many cuttings were heavily rooted as when treated with indolebutyric. Treatment with naphthaleneacetic acid at 1000 p.p.m. induced some inhibition in top growth. Cuttings treated with naphthalene acetamide developed shorter and thicker roots than those treated with indolebutyric or naphthaleneacetic acid, and there were not as many heavily rooted cuttings, although more roots were formed than by the controls. Top growth and root production of material treated with naphthoxyacetic at 200 and 1000 p.p.m. were inhibited. At 50 p.p.m. fewer roots were produced than in the controls.

Mixtures of several growth substances have been shown to be more effective for root formation in cuttings than equivalent concentrations of the individual substance (1). Cuttings of goldenrod treated with a mixture of equal parts of naphthaleneacetic acid, naphthalene acetamide, naphthoxyacetic and indolebutyric acids at a total concentration of 50 or 200 p.p.m. produced more roots than the controls, but at 1000 p.p.m. top growth was considerably less. The mixture was not as effective in these tests as was indolebutyric acid.

Stems

As in the case of stolon cuttings, sand and soil were both suitable substrates for rooting. Growth after rooting had begun was more extensive in soil than in sand.

The length of the cutting taken from young and mature stems was not so important a factor as in the rooting of old, over-mature stem cuttings. Three-inch and 6-inch cuttings of young and mature stems rooted readily and profusely, regardless of treatment or diameter size. In the old, over-mature stems, 6-inch cuttings produced more roots than 3-inch ones. However, only about 5% of the cuttings from the over-mature stems rooted; most of them were of larger diameter and taken from the base of the stem.

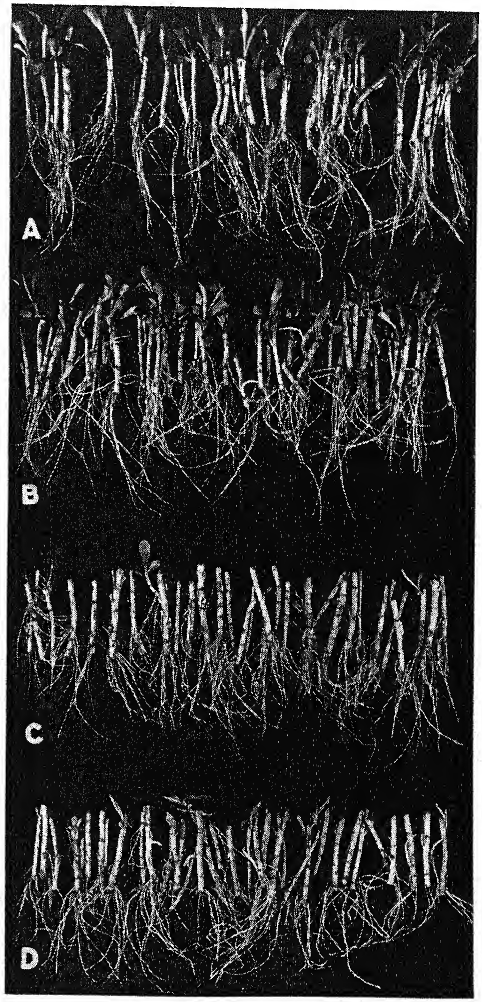

FIG. 3.—Stolon cuttings of goldenrod soaked in solutions of ibc for 3 hours. *A*, water only; *B*, 10 p.p.m.; *C*, 100 p.p.m.; *D*, 1000 p.p.m.

Roots were produced by a much greater percentage of cuttings from stems that still had their leaves than by those

from old, over-mature stems without leaves. A greater number and quantity of roots were found on the cuttings from the young and mature stems. Cuttings in rooting (table 2, figs. 5, 6). Cuttings from the region nearest the tip in the young stems produced more roots than did those from the middle of the stem or

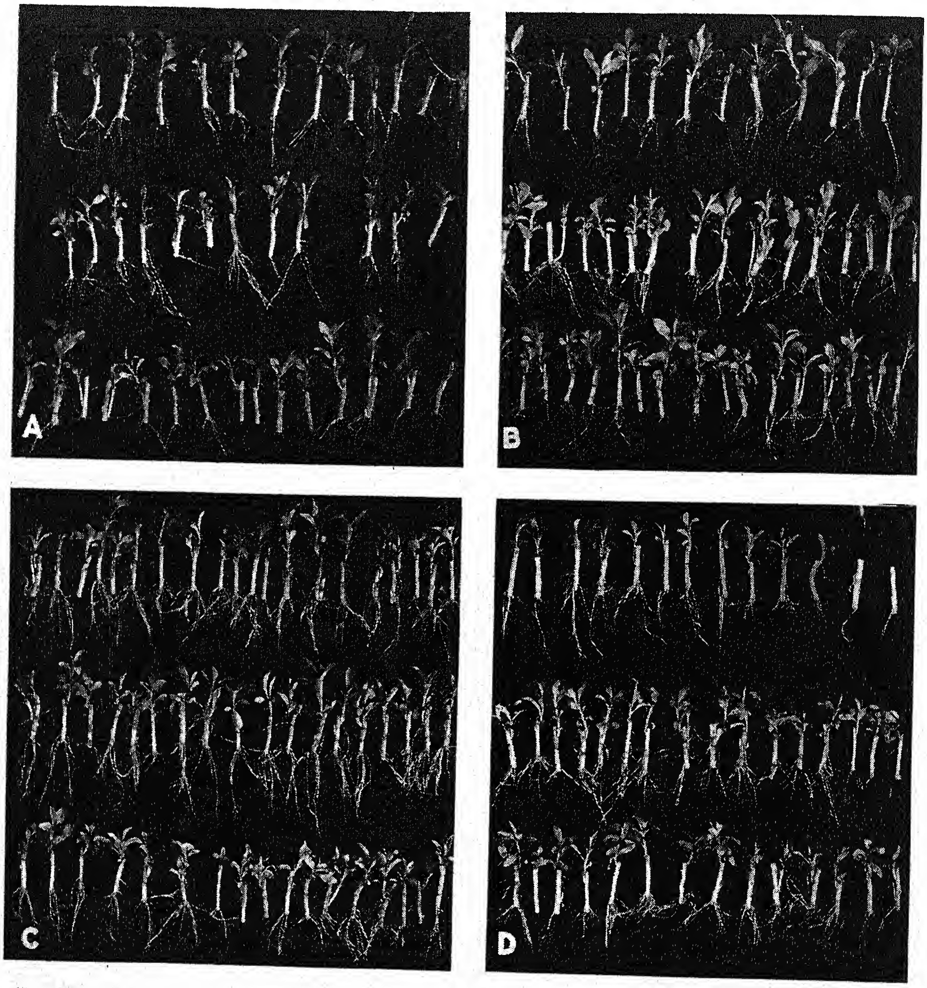

FIG. 4.—Rooting response of stolon cuttings of goldenrod to growth regulators dispersed in talc powder. Top row in each group 1000 p.p.m., middle row 200 p.p.m., bottom row 50 p.p.m. *A*, naphthaleneacetic acid (nac); *B*, naphthalene acetamide (nad); *C*, indolebutyric acid (ibc); *D*, mixture of ibc, nac, nad, and naphthoxyacetic acid (noac), total of 50 p.p.m.

from young stems 1–2 feet tall produced more roots than those from mature stems 2–5 feet tall, but the difference was not great.

The region of the stem from which cuttings were made was very important from the base, and cuttings from the middle region produced more roots than did those from the base. The same results were obtained with mature stems 2–5 feet tall. In the over-mature stems in which the leaves had died, the re-

TABLE 1

RESPONSE OF STOLONS OF GOLDENROD CUT 3 INCHES LONG TO GROWTH SUBSTANCES

Lot	Time of application	Treatment: Preparation	Treatment: Concentration (p.p.m.)	Time in solution (hours)	Percentage cuttings showing various degrees of rooting: None	Few	Medium	Heavy	Remarks
I. Rooted in sand at 65°–75°									
A.	Jan.	Water: Control	....	18	10	90	0	0	Poor rooting but good top growth
B.	"	Ibc*	50	18	20	80	0	0	Marked inhibition of tops
C.	"	Ibc	10	18	0	40	60	0	Marked improvement over controls
D.	"	Mixture of ibc, nac, nad, noac	50	18	40	60	0	0	Top inhibition
E.	"	"	10	18	20	80	0	0	Comparable with controls
II. Rooted in soil at 65°–75°									
A.	Jan.	Talc dust: Control	....	......	10	70	20	0	....
B.	"	Nac	1000	......	0	100	0	0	Some top inhibition
C.	"	Nac	200	......	0	20	60	20	....
D.	"	Nac	50	......	0	70	30	0	....
E.	"	Nad	1000	......	0	100	0	0	....
F.	"	Nad	200	......	0	10	60	30	....
G.	"	Nad	50	......	0	60	40	0	....
H.	"	Noac	1000	......	70	30	0	0	Too concentrated
I.	"	Noac	200	......	20	70	10	0	....
J.	"	Noac	50	......	0	100	0	0	....
K.	"	Mixture	1000	......	20	80	0	0	....
L.	"	Mixture	200	......	0	0	80	20	....
M.	"	Mixture	50	......	0	0	80	20	....
N.	"	Ibc	1000	......	0	0	40	60	....
O.	"	Ibc	200	......	0	0	60	40	....
P.	"	Ibc	50	......	10	70	20	0	....
III. Rooted in sand at various temperatures									
A. (60° F.)	Feb.	Water: Control	....	3	60	40	0	0	....
B. (70° F.)	"	Control	....	3	30	50	20	0	....
C. (80° F.)	"	Control	....	3	30	20	50	0	....
D. (60° F.)	"	Ibc	50	3	5	45	50	0	....
E. (70° F.)	"	Ibc	50	3	5	10	55	30	....
F. (80° F.)	"	Ibc	50	3	5	5	70	20	....

* Ibc, indolebutyric acid; nac, naphthaleneacetic acid; nad, naphthalene acetamide; noac, naphthoxyacetic acid.

TABLE 2

RESPONSE OF STEMS OF GOLDENROD TO GROWTH SUBSTANCES

Lot	Time of application	Treatment: Preparation	Treatment: Concentration (p.p.m.)	Time in solution (hours)	Percentage cuttings showing various degrees of rooting: None	Few	Medium	Heavy
I. Young stems about 3 inches tall cut at ground level and rooted in sand at 80°–85°								
		Water:						
A.	Feb.	Control	....	3	20	46	26	8
B.	"	Ibc*	100	3	20	8	33	39
C.	"	Ibc	50	3	35	35	10	20
D.	"	Ibc	25	3	37	32	18	13
E.	"	Nac	100	3	13	20	20	47
F.	"	Nac	50	3	6	39	6	49
G.	"	Nac	25	3	28	6	40	26
H.	"	Noac	100	3	....	....	....	....
I.	"	Noac	50	3	29	0	23	48
J.	"	Noac	25	3	48	6	0	46
II. Young stems about 1 foot tall cut into 3-inch lengths and rooted in sand at 80°–85°								
		Water:						
A. Base	Feb.	Control	....	....	0	44	56	0
Middle	"	Control	....	....	0	55	45	0
Tip	"	Control	....	....	0	33	58	9
B. Base	"	Ibc	25	3	0	61	39	0
Middle	"	Ibc	25	3	0	25	50	25
Tip	"	Ibc	25	3	0	12	33	55
C. Base	"	Ibc	50	3	0	56	44	0
Middle	"	Ibc	50	3	0	29.5	70.5	0
Tip	"	Ibc	50	3	0	22	26	52
III. Young stems 1 foot tall cut into two pieces in sand at 80° with no shade								
		Talc dust:						
A. Base	April	Control	....	....	0	0	92	8
Tip	"	Control	....	....	0	0	40	60
B. Base	"	Nad	50	....	0	0	72	18
Tip	"	Nad	50	....	0	0	56	44
C. Base	"	Mixture	50	....	0	0	80	20
Tip	"	Mixture	50	....	0	0	40	60
D. Base	"	Nac	50	....	0	0	84	16
Tip	"	Nac	50	....	0	0	44	56

* Ibc, indolebutyric acid; nac, naphthaleneacetic acid; noac, naphthoxyacetic acid; nad, naphthalene acetamide.

TABLE 2—*Continued*

Lot	Time of application	Treatment: Preparation	Treatment: Concentration (p.p.m.)	Time in solution (hours)	Percentage cuttings showing various degrees of rooting: None	Few	Medium	Heavy
IV. Young stems approximately 3 feet tall and about $\frac{3}{8}$ inch in diameter at base, cut into 6-inch lengths and rooted in sand at 90° with shade								
		Talc dust:						
A. Base	April	Control	....	....	0	45	50	0
Middle	"	"	....	....	0	35	50	15
Tip	"	"	....	....	0	20	50	30
B. Base	"	Ibc	50	....	0	44	56	0
Middle	"	"	50	....	0	9.5	70	20.5
Tip	"	"	50	....	0	12	52	36
C. Base	"	Mixture	50	....	0	16	36	48
Middle	"	"	50	....	0	8.1	46.6	45.3
Tip	"	"	50	....	0	0	52	48
D. Base	"	Nac	50	....	0	72	28	0
Middle	"	"	50	....	0	49	33	18
Tip	"	"	50	....	0	0	36	64
V. Young stems 1 foot tall, cut into 4-inch pieces and grown in sand at 80°								
		Water:						
A.	April	Control	....	3	0	40	52	8
B.	"	Nad	25	3	0	34	44	22
C.	"	Nad	50	3	0	24	48	28
D.	"	Nac	25	3	0	26	60	14
E.	"	Nac	50	3	0	36	52	12
F.	"	Noac	25	3	0	50	42	8
G.	"	Noac	50	3	0	48	20	32
H.	"	Ibc	25	3	0	26	48	26
I.	"	Ibc	50	3	0	28	22	50
VI. Mature stems 4–5 feet tall, cut into 6-inch lengths examined after 16 days								
		Water:						
A. Base	May 29	Control	....	3	9.5	40	51.5	0
Middle	"	"	....	3	12	34	40	14
Tip	"	"	....	3	30	55	15	0
B. Base	"	Ibc	50	3	0	0	46	54
Middle	"	"	50	3	0	42	12	46
Tip	"	"	50	3	6	18	6	70

verse was the case; the cuttings from the basal region produced more roots than those from the middle or top region. were dipped in talc. Three hours was sufficient time for soaking the young and mature stem cuttings in a solution.

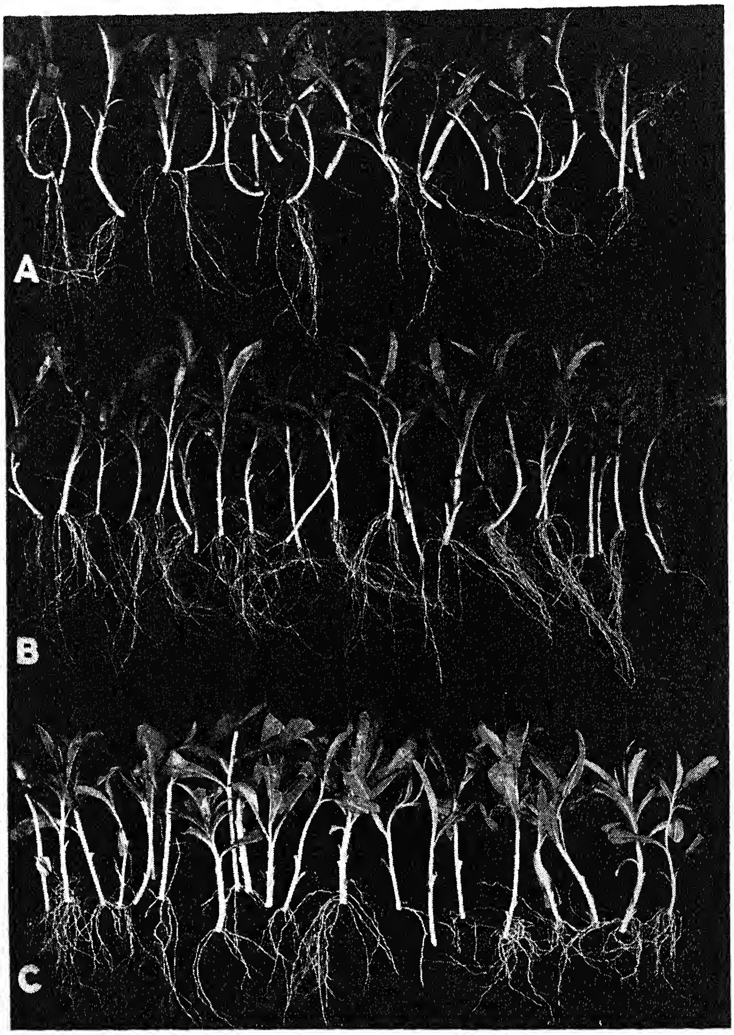

FIG. 5.—Cuttings of young goldenrod stems soaked in solutions of ibc for 3 hours. *A*, water only; *B*, 25 p.p.m.; *C*, 100 p.p.m.

Data for the over-mature cuttings were not included.

In contrast to stolon cuttings, stem cuttings produced more roots when they were soaked in a solution than when they

In a number of experiments on young stems, some of which are recorded in table 2, it was found that, although the response of young stem cuttings to growth substances varied with each ex-

periment, cuttings soaked in indolebutyric and naphthaleneacetic acid consistently produced more roots than the controls.

From over-mature stems a greater number of cuttings rooted when soaked in naphthaleneacetic or indolebutyric acid at 100 p.p.m. for 18 hours than without treatment. A greater amount of rooting occurred on the treated cuttings. Although data are not here provided, only a small percentage of over-mature stems rooted, and they required longer for rooting than did young or mature stems.

Talc dust was a satisfactory carrier for the growth substances but less so than water. Cuttings treated with any of the growth substances at 50 p.p.m. in talc produced more roots than did the controls (table 2).

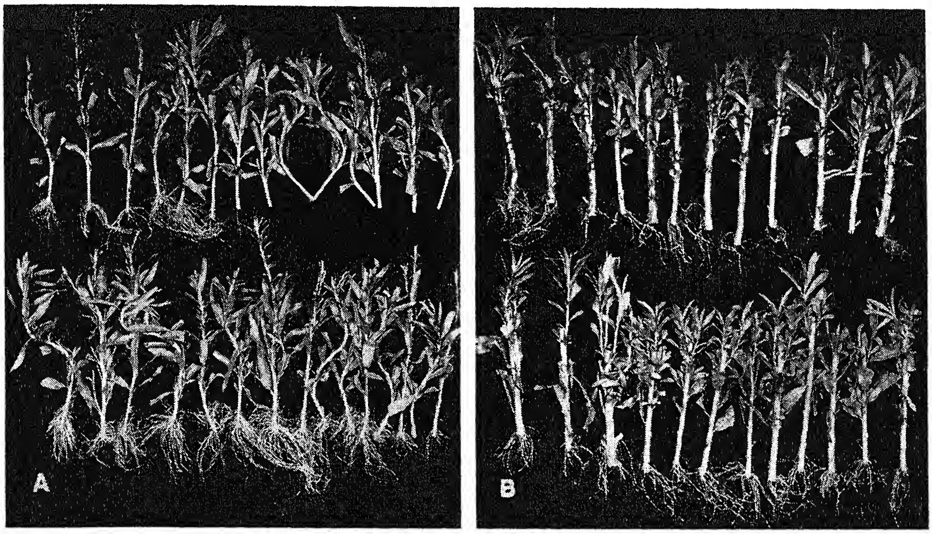

FIG. 6.—Rooting of tip and basal sections of mature goldenrod stems. *A*, tip region: upper row, controls; lower row, soaked in aqueous solution ibc, 50 p.p.m., for 3 hours. *B*, basal region: upper row, controls; lower row, aqueous solution ibc, 50 p.p.m.

Summary

STOLONS

1. Stolon cuttings of goldenrod soaked in aqueous solutions of several growth substances at concentrations of 10 to 100 p.p.m. for 18 hours were inhibited in top growth. Some of the cuttings rotted following this long treatment. Soaking for 3 hours in indolebutyric-acid solution at 10 or 50 p.p m. gave some increase in rooting over the controls.

2. Cuttings dusted with indolebutyric, naphthaleneacetic, naphthoxyacetic acids, or naphthalene acetamide, or a mixture of these four, dispersed in powdered talc produced more roots than did those without treatment, except when the highest concentrations were used.

3. When the temperature of the sub-

strate was 70°–80° F., the greatest number and quantity of roots were formed.

4. Regardless of treatment, stolons cut into 3-inch pieces produced many roots and heavy top growth. Those cut into $\frac{1}{2}$-inch pieces produced few roots and only 50% of the pieces rooted; top growth was slight.

STEMS

5. Cuttings from young stems produced a more vigorous root and top system than did cuttings from more mature stems. Cuttings from the tips of both young and mature stems rooted more abundantly than did cuttings from the base of the stems.

6. Only a small percentage of cuttings from old, over-mature, leafless stems formed roots, and these were not numerous.

7. Cuttings soaked in aqueous solutions of indolebutyric acid at 25, 50, or 100 p.p.m. for 3 hours produced more roots than did the controls.

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LITERATURE CITED

1. HITCHCOCK, A. E., and ZIMMERMAN, P. W., Effects obtained with mixture of root inducing and other substances. Contrib. Boyce Thompson Inst. **11**: 143–160. 1940.
2. MITCHELL, J. W., and RICE, RUBY R., Plant-growth regulators. Misc. Publ. U. S. Dept. Agr. No. 495. 1942.

STRUCTURE AND DEVELOPMENT OF SYMPHYOGYNA BRASILIENSIS

ARTHUR W. HAUPT

Introduction

According to SCHIFFNER (10), *Symphyogyna* includes 27 species. A few of these are found in tropical parts of the Northern Hemisphere, but most of them occur only south of the Equator, where they range from tropical to antarctic regions. STEPHANI (11) recognized a greater number of species than SCHIFFNER, fifty-six in all, to which EVANS (2, 3) has added several new ones from America. Although the classification of the anacrogynous Jungermanniales is a matter of considerable difference of opinion, especially regarding the number and delimitation of the families, there is no doubt as to the closeness of relationship between *Symphyogyna* and *Pallavicinia*. All authorities place them in the same family, which has been variously designated as the Dilaenaceae, Pallaviciniaceae, Blyttiaceae, or Leptotheceae.

Symphyogyna brasiliensis Nees is widely distributed in tropical America, ranging from Mexico to Bolivia and Brazil. Material for the present study was collected by the writer in Costa Rica during July and August of 1940. This species, often associated with *S. brogniartii* Mont., was found in several parts of the mountains lying both to the north and south of the city of San José. Specific localities are given elsewhere (7). The most vigorous plants were found in densely wooded ravines, growing in shade on rich moist humus.

Most of the material was fixed in Randolph's modification of Navashin's fluid, which was allowed to act for 2–3 days. Some material was also fixed in a 3.5% solution of glacial acetic acid to which had been added 0.5 gm. of chromic-acid crystals to each 100 cc. of solution. This was allowed to act for only 24 hours. Both fluids gave thoroughly satisfactory results. After washing for about 12 hours or more, the material was transferred to a 5% solution of formalin. Several months later it was prepared for imbedding in paraffin by the ethyl alcohol-xylol technique, sectioned at 8 μ, and stained in iron-alum haematoxylin, followed by either erythrosin or orange G.

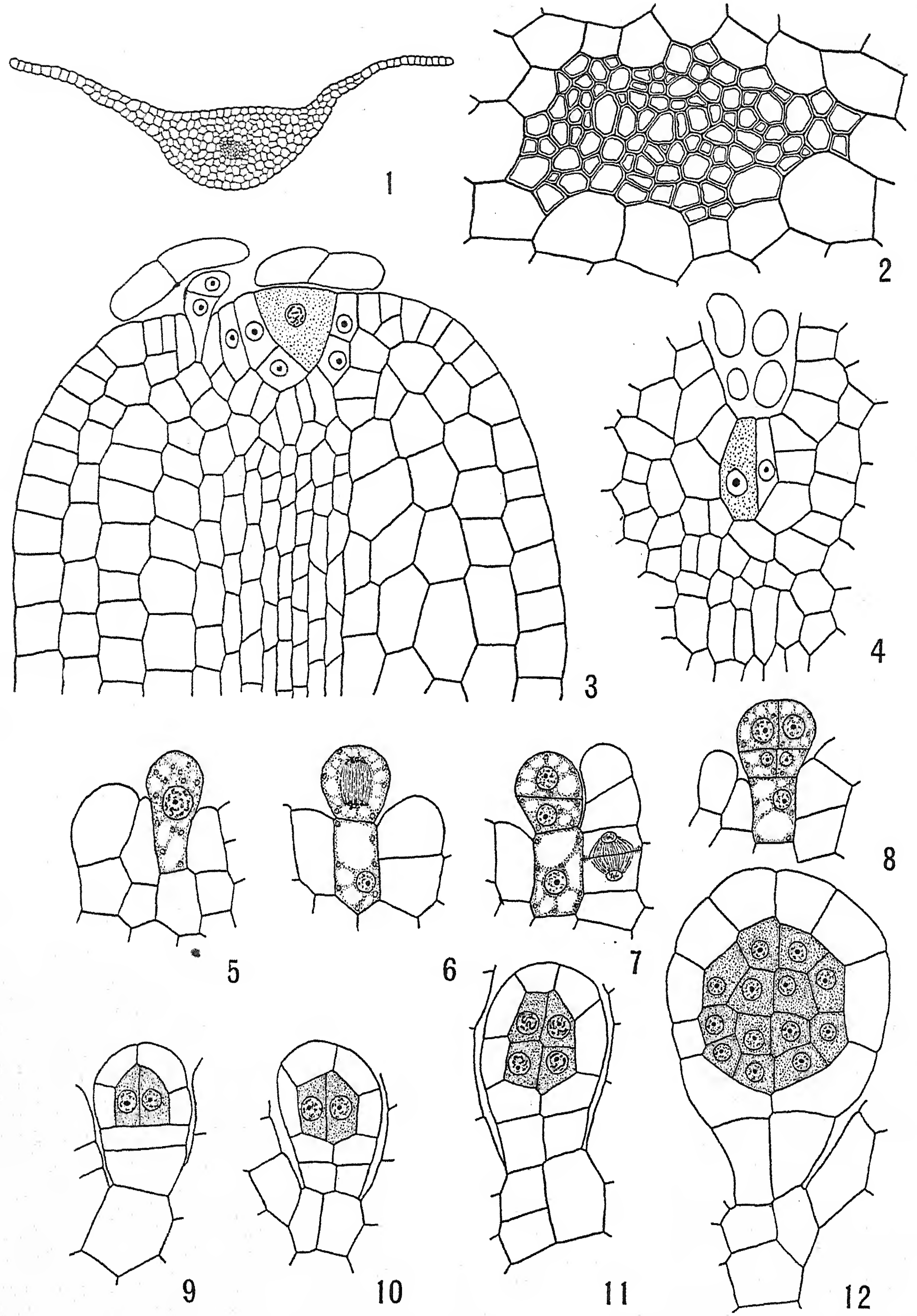
Previous morphological work on *Symphyogyna* has been done by LEITGEB (8), who studied *S. subsimplex*, *S. rhizoloba*, and *S. sinuata*, and by MCCORMICK (9), who studied *S. aspera*.

Observations

THALLUS

S. brasiliensis is one of a group of species having a ribbon-like prostrate thallus bearing rhizoids on its lower surface. Except when young, however, the tips of the thallus tend to become ascending and, not being in contact with the substratum, fail to produce rhizoids. This is especially true of plants growing in crowded mats. The thalli are light green and often more or less tinged with reddish purple. Plants growing in open situations are more likely to show this reddish pigmentation than those living in moist shady places.

The thallus consists of a distinct midrib and lateral wings, the latter gradually thinning to a single layer of cells (fig. 1). The wings are unistratose for one-half or more of their width. The upper surface of the thallus is nearly flat, the midrib projecting below and bearing long colorless rhizoids where it touches the soil. Ventral scales are lacking. The extreme base of the thallus is without wings; it is stalklike and only 2–3 mm. long. The entire thallus is generally 10–20 mm. long, but under optimum conditions it may reach a length of 35–45 mm. or more.



FIGS. 1–12.—Figs. 1–4, thallus: Fig. 1, transverse section. Fig. 2, central strand of conducting cells. Fig. 3, median vertical section of apex showing apical cell and young archegonium. Fig. 4, horizontal section of same. Figs. 5–12, development of antheridium: Fig. 5, antheridium initial. Fig. 6, formation of basal cell and division of outer cell. Fig. 7, formation of primary antheridial cell and primary stalk cell. Fig. 8, appearance of vertical walls. Figs. 9, 10, appearance of periclinal walls, differentiating jacket, and spermatogenous cells. Figs. 11, 12, later stages. Fig. 1, ×25; figs. 2–4, ×350; others ×500.

The male plants average 2–3 mm. in width but are occasionally up to 4 mm. wide. The female plants are larger, averaging 4–5 mm., or sometimes as much as 6 mm., in width. The margins of the thallus are slightly undulate in the vertical plane and, in the material studied, are entirely without teeth or lobes. Most of the plants are unbranched, but many show a single dichotomy; a few plants exhibit two or even three dichotomies. Occasionally plants with ventral adventitious branches are seen, these narrowing to a stalklike base that arises from alongside the midrib.

The midrib is traversed by a central strand of narrow, elongated conducting cells with thick pitted walls and pointed ends (fig. 2). They are differentiated immediately behind the apical cell (fig. 3). The conducting strand, as seen in cross section, comprises about eighty cells. It lies at or slightly below the center of the midrib. In *Pallavicinia*, which has the same kind of conducting cells as *Symphyogyna*, Tansley and Chick (12) have demonstrated, by means of eosin solutions, that they conduct water. In *S. aspera* McCormick (9) has shown that their walls are composed of pectose.

All cells in the younger portion of the thallus (except those forming the conducting strand) contain chloroplasts, while in older parts many starch grains are frequently present in cells below the epidermis. Intracellular fungi inhabit the thallus, but not as a rule in the portion that is free from the substratum.

The apex of the thallus is deeply notched. It contains an apical cell that, in the material studied, is of the cuneate type. As seen in median vertical section, the apical cell is broadly triangular in outline, cutting off segments both above and below (fig. 3). In a horizontal section the apical cell appears rectangular, or nearly so, and cuts off segments to the right and left (fig. 4). Numerous sections cut in this plane were examined and in none was the apical cell pointed at the base, as would be true of a dolabrate apical cell. In a transverse section the apical cell also appears rectangular. Short 2-celled mucilage hairs arise in the growing notch both above and below the apical cell.

Although an apical cell of the dolabrate type is characteristic of the majority of the anacrogynous Jungermanniales, several exceptions have been noted, as in *Pellia*, *Blasia*, and *Moerckia*. In the three species of *Symphyogyna* that he studied, Leitgeb (8) found a dolabrate apical cell. In *S. aspera* McCormick (9) found two types of apical cell, the dolabrate and the cuneate, each characteristic of plants collected in two different places in Mexico. In *Pallavicinia* a dolabrate apical cell has been seen by Campbell and Williams (1), who investigated three different species, and by Haupt (4), who studied *P. lyellii*.

Sex organs

Like other species of the genus, *S. brasiliensis* is dioecious, the male plants being more slender than the female. The sex organs, always borne on the main thallus, are dorsal in position, occurring along the midrib. The antheridia are in long crowded groups that often extend almost the entire length of the thallus. They form a band, about 1 mm. wide, in which they are scattered irregularly, although often appearing to be arranged in three to five indefinite rows. The antheridia lying along the center of the midrib point forward, while those along its sides point diagonally outward. They are not sunken in the thallus, but each is inclosed by a scalelike posterior involucre that arches toward the apex of the thallus.

The archegonia occur in isolated groups lying directly upon the midrib. These groups are generally 4–6 mm. apart. The number of archegonia in a group is not so high as in certain other species, such as *S. brogniartii*, but reaches about twelve. The necks of the archegonia lie parallel with the surface of the thallus and point forward. According to EVANS (3), "the female inflorescences [of *S. brasiliensis*] are borne singly or in small numbers, three on one thallus being the highest number observed." The writer has seen many plants with more than three archegonial groups. One plant, unbranched and measuring 42 mm. in length, had ten archegonial groups, equally spaced, extending along its entire length.

Each group of archegonia, slightly raised on a pad, is covered by a flaplike posterior involucre that is open in front but attached to the thallus behind and on the sides. The involucre is partially dissected into two or three segments, each of which may be somewhat laciniate.

ANTHERIDIUM

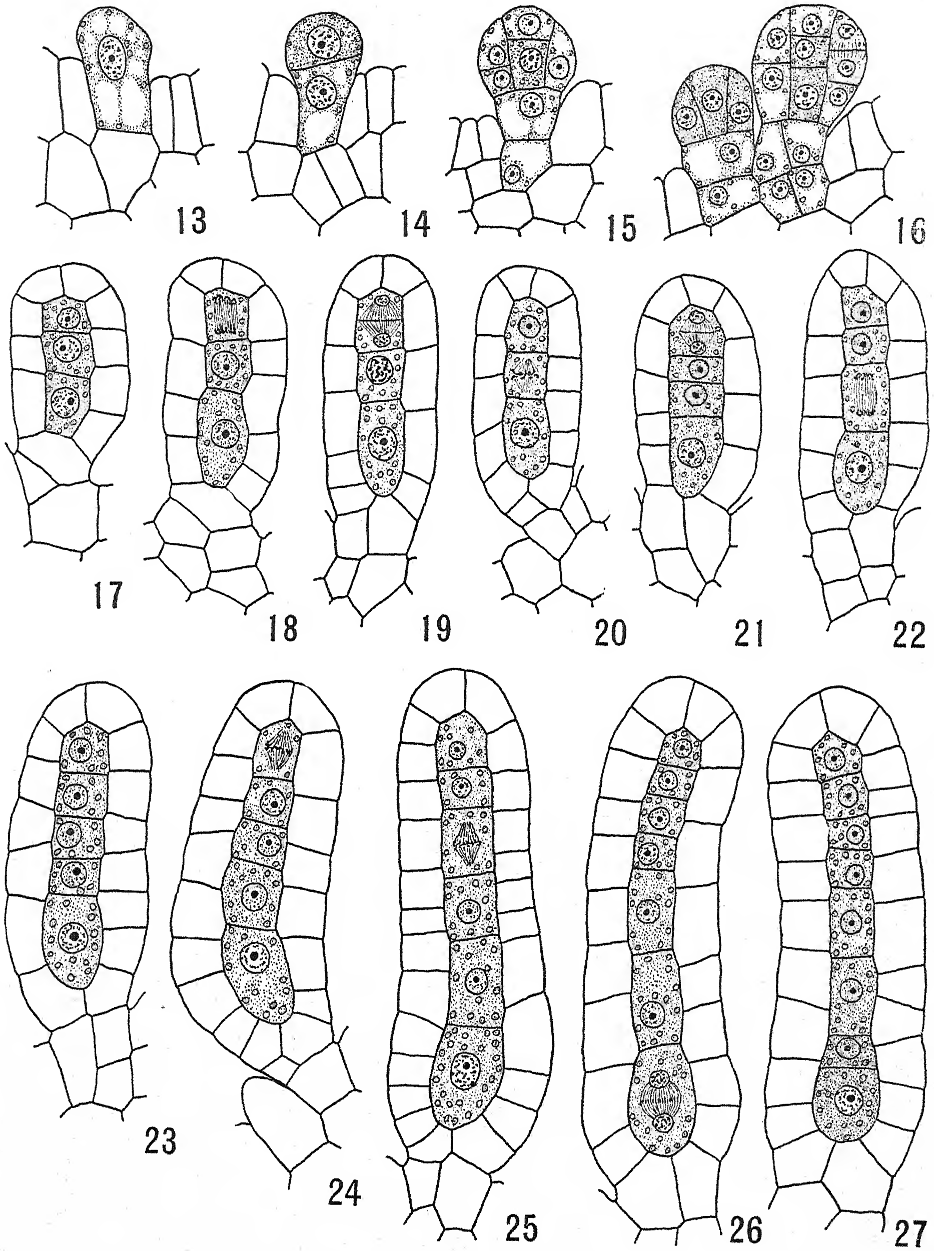
The antheridia arise close to the apical cell and develop in acropetal succession from its immediate dorsal segments, generally from the third or fourth one. The development is similar to that previously described for *Pallavicinia* (4) and *Fossombronia* (5, 6). The initial is papillate and, by a transverse division, gives rise to an outer free cell and a basal imbedded cell (figs. 5, 6). The outer cell undergoes another transverse division into two approximately equal segments, the upper one being the primary antheridial cell and the lower one the primary stalk cell (figs. 6, 7). The next division, a median vertical one in the antheridial cell, is followed by a division in the stalk cell that is usually vertical but may be transverse (fig. 8).

Generally two additional divisions complete the stalk, while a periclinal wall, intersecting the median wall, is now established in each of the two cells derived from the primary antheridial cell. Two additional periclinal walls, coming in at right angles to the first periclinal ones, intersect both these and the median wall. As a result four outer sterile cells are separated from two central spermatogenous cells, as in all the Jungermanniales (figs. 9, 10). The outer cells increase in number by anticlinal divisions and give rise to a single-layered sterile jacket, while the spermatogenous cells multiply by divisions in all three planes (figs. 11, 12). The cells of the sterile jacket at this and subsequent stages contain chloroplasts, but the spermatogenous cells do not. The antheridium soon assumes a spherical form, its stalk remaining very short. Later stages resemble those of other anacrogynous Jungermanniales.

At a very early stage in the development of the antheridium, the involucre appears as an upgrowth of the thallus immediately behind it (fig. 7). It consists of a single layer of cells that soon overarches the antheridium, finally reaching a length of about 7–9 cells.

ARCHEGONIUM

The archegonia originate very close to the apical cell, arising, as MCCORMICK (9) has observed, from approximately the fourth dorsal segment (fig. 3). Their development is not strictly acropetal, however, since young archegonia may continue to arise among the older ones, as in *Pallavicinia* (4). The papillate initial divides transversely to form an outer free cell and a basal imbedded cell (figs. 13, 14). The usual three intersecting verti-



FIGS. 13–27.—Early stages in development of archegonium: Fig. 13, archegonium initial. Fig. 14, first division of initial. Fig. 15, formation of central cell and cover cell. Fig. 16, formation of primary axial cell and primary wall cells (left) and of primary ventral cell and primary neck canal cell (right). Figs. 17–25, formation of neck canal cells. Figs. 26, 27, differentiation of ventral canal cell and egg from primary ventral cell. ×440.

cal walls now appear in the outer cell, cutting off three primary jacket cells from the primary axial cell, while a transverse division occurs in the basal one (fig. 16). The cover cell and central cell are now differentiated from the primary axial one (fig. 15), the central cell soon giving rise to the primary neck canal cell and primary ventral one (fig. 16). The primary neck canal cell divides transversely to form an upper and a lower segment, while—either at this stage or at the one immediately following—the cover cell divides by a vertical wall (fig. 17).

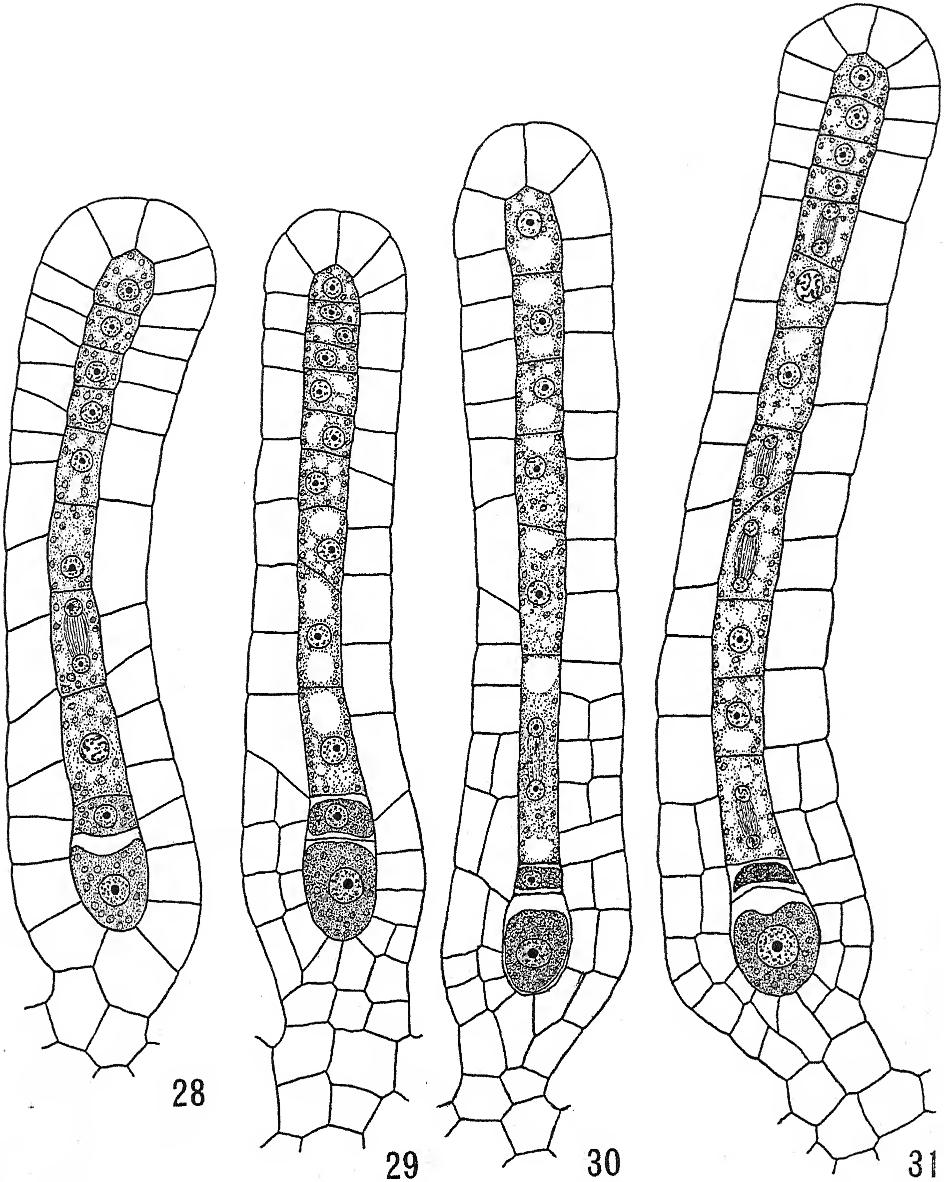
Each of the two cells derived from the primary neck canal cell again divides transversely, the first division occurring either in the upper cell (figs. 18, 19, 22) or in the lower one (figs. 20, 21). Sometimes the division of the second cell is begun before that of the first one is completed (fig. 19). The young archegonium now consists of a primary ventral cell, four neck canal cells, and a single layer of outer cells forming the sterile jacket (fig. 23). As a rule, each of the two uppermost neck canal cells undergoes another transverse division (figs. 24, 25), and then division of the primary ventral cell takes place, differentiating the ventral canal cell and egg (figs. 26, 27). The division of the primary ventral cell may occur somewhat earlier, however, one archegonium having been seen with an egg, a ventral canal cell, and five neck canal cells. In this case only the uppermost of the four original neck canal cells had again divided.

As the protoplast of the egg begins to round off, the neck of the archegonium continues to increase in length and the neck canal cells continue to increase in number (figs. 28–31). At the same time the venter becomes 2-layered. The upper neck canal cells divide more rapidly than the lower ones, and although there is no indication that the divisions occur in strict acropetal sequence, there is a tendency in this direction. The archegonium represented by figure 31 is 350 μ long but has not yet reached its full length, which measurement of slightly older archegonia shows to be approximately 500 μ.

The final divisions of the neck canal cells are unaccompanied by cross walls, so that many or all of them are binucleate. The number of neck canal nuclei may ultimately reach eighteen or twenty. The greatest number of neck canal cells counted by McCormick (9) was thirteen, but she says nothing about any of them being binucleate. The neck becomes curved and frequently twisted, so that it is not always easy to determine from a series of consecutive sections the exact number of neck canal cells and nuclei finally present.

At all developmental stages the venter is slender, only slightly exceeding the neck in diameter. The ventral canal cell is relatively large. It becomes very dense and begins to break down before the neck canal cells show any evidence of disintegration. At all stages the cells of both the axial row and jacket contain numerous plastids. The neck canal cells are surrounded by five rows of neck cells. The involucre begins to develop behind the archegonia as soon as two or three of them have arisen from the immediate dorsal segments of the apical cell. At first it is only one layer of cells thick, but later the basal half becomes 3- or 4-layered. Around and among the archegonia, as noted by Leitgeb (8), are numerous 2-celled, clavate mucilage hairs similar to those found at the apex of the thallus.

In *Pallavicinia lyellii* the writer (4) found that about ten neck canal cells are formed before the ventral canal cell and egg are differentiated, but that addition-



28 29 30 31

FIGS. 28–31.—Older stages in development of archegonium, showing rounding off of ventral canal cell and egg and formation of additional neck canal cells. ×440.

al neck canal cells may arise later. Some of these are binucleate, the total number of nuclei sometimes reaching eighteen. The venter becomes 2-layered previous to fertilization, about when the ventral canal cell begins to disorganize. In both *Pallavicinia* and *Symphyogyna* the belated division of the ventral cell to form the ventral canal cell and egg and the subsequent increase in the number of neck canal cells may be regarded as primitive features.

Sporophyte

Not enough stages in embryogeny were found to make possible a detailed account. The few stages that were observed indicate that the sequence is essentially the same as in *Symphyogyna rhizoloba*, as presented by Leitgeb (8), and *S. aspera*, as given by McCormick (9).

The first division of the fertilized egg, which is transverse, is followed by a similar division that, according to Leitgeb, occurs in the epibasal segment. Thus a row of three superimposed cells is formed. As a result of the establishment and functioning of a dolabrate apical cell, the embryo soon becomes elongated. The uppermost part is most active in cell division, the middle part in elongation, while the innermost cell seems to divide only a very few times. No evidence has been seen of the formation of a haustorium-like appendage to the foot, such as is present in *Pallavicinia*. In *S. aspera* (9) three wall layers are formed following delimitation of the amphithecium from the endothecium. The cell walls of the outer layer later thicken uniformly without forming semiannular bands, and the two inner layers do not entirely disappear. As is well known, a pseudoperianth is not developed in *Symphyogyna*, but the calyptra becomes thick and fleshy, being many-layered almost to the apex and with a cluster of about twelve unfertilized archegonia at its summit. In *S. brasiliensis* the calyptra becomes 4–5 mm. long and approximately 0.8 mm. in diameter. Normally only one sporophyte develops in each archegonial group.

Formation of the spore mother cells has been thoroughly studied by McCormick (9). The sporogenous tissue, differentiated relatively late, is at first composed of uniform cells. The cells that are to become elaters elongate without further division, while those that ultimately give rise to spore mother cells divide several times. The protoplasts of the latter withdraw from their cell walls, which break down to form a gelatinous matrix. The mother cells assume an irregular amoeboid form, finally becoming prominently 4-lobed. The process of lobing is accompanied by the formation of large vacuoles.

The mature capsule is long-stalked and cylindrical. Its wall is composed of two layers of cells, the cell walls of the outer layer being uniformly thickened. At the apex of the capsule the wall is four or five layers thick, forming a prominent sterile cap. This bears no relation to the elaters. The foot is slender and rounded. It is not sharply differentiated from the seta. The elaters have two spiral bands of thickening and, according to Evans (3), vary in length from 100 to 800 μ. They fall away in dehiscence. The mature spores are 22–26 μ in diameter, their wall being covered with low ridges running in all directions. The capsule reaches a length of 2.5–3.0 mm. and a diameter of 0.6–0.8 mm. Dehiscence takes place by means of four valves that remain united at the apex.

Summary

1. The thallus of *Symphyogyna brasiliensis* grows by means of a cuneate api-

cal cell. Branching is generally apical but sometimes ventral and adventitious.

2. The thalli are strictly dioecious, the male plants being smaller than the female.

3. The antheridia are crowded and scattered irregularly in a long band lying above the midrib, each inclosed by a posterior involucre.

4. Development of the antheridium follows the general pattern of the anacrogynous Jungermanniales.

5. The archegonia are in isolated dorsal groups of about twelve, each group on a pad and covered by a posterior involucre.

6. In the development of the archegonium, generally six neck canal cells are formed before the ventral canal cell and egg are differentiated.

7. The number of neck canal cells is later increased, often up to twelve or fifteen; since some of these are binucleate, there are often as many as twenty neck canal nuclei.

8. The venter becomes 2-layered before fertilization.

9. The embryogeny follows the sequence of stages previously described for other species.

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LITERATURE CITED

1. CAMPBELL, D. H., and WILLIAMS, FLORENCE, A morphological study of some members of the genus *Pallavicinia*. Leland Stanford Junior Univ. Publ. Univ. Series. pp. 44. 1914.
2. EVANS, A. W., The lobate species of *Symphyogyna*. Trans. Conn. Acad. Arts Sci. **27**:1–50. 1925.
3. ———, A further study of the American species of *Symphyogyna*. Trans. Conn. Acad. Arts Sci. **28**:295–354. 1927.
4. HAUPT, A. W., A morphological study of *Pallavicinia lyellii*. BOT. GAZ. **66**:524–533. 1918.
5. ———, Life history of *Fossombronia cristula*. BOT. GAZ. **69**:318–331. 1920.
6. ———, Studies in Californian Hepaticae. II: *Fossombronia longiseta*. BOT. GAZ. **88**:103–108. 1929.
7. ———, Notes on some Hepaticae of Costa Rica. Bryologist **45**:138–146. 1942.
8. LEITGEB, H., Untersuchungen über die Lebermoose. III: Die frondosen Jungermannien. Leipzig. 1877.
9. MCCORMICK, FLORENCE A., A study of *Symphyogyna aspera*. BOT. GAZ. **58**:401–418. 1914.
10. SCHIFFNER, V., Hepaticae in ENGLER and PRANTL'S Natürlichen Pflanzenfamilien **1**^{3}:1–144. 1909.
11. STEPHANI, F., Species Hepaticarum. Geneva. 1900–1924.
12. TANSLEY, A. G., and CHICK, EDITH, Notes on the conducting tissue system in Bryophyta. Ann. Bot. **15**:1–38. 1901.

ORIGIN AND DEVELOPMENT OF EMBRYOS IN CERTAIN APOGAMOUS FORMS OF DRYOPTERIS[1]

ROBERT E. DUNCAN

The first description of apogamy in *Dryopteris* is that of DE BARY (1) who reported that prothallia of *Aspidium filix-mas* var. *cristatum* form embryos in the same manner as prothallia of *Pteris cretica* var. *albo-lineata*. The second study is that of LANG (7) who listed *Nephrodium propinquum* var. *cristatum* and *N. filix-mas* var. *cristatum* as forming sexual embryos and *N. pseudo-mas*, *N. pseudo-mas* var. *cristatum*, *N. pseudo-mas* var. *polydactyla* Wills, *N. pseudo-mas* var. *polydactyla* Dadds, and *N. filix-mas* var. *polydactylum cristatum* as being "directly" apogamous. MANTON (8) pointed out that these species belong to *Dryopteris filix-mas* (L.) Schott., within which she distinguished three groups corresponding to the three species WOLLASTON (11) separated out of the *filix-mas* complex. MANTON found *Lastrea propinqua* Woll. to have a sporophytic chromosome number of about 80 and sexual reproduction; *L. filix-mas* about 160 chromosomes and sexual reproduction; and apogamous races of *L. pseudo-mas* Woll. about 80, 120, and 160 chromosomes. LANG (7) listed a number of forms of *Aspidium* and *Nephrodium* which are normally sexual but whose prothallia, when fertilization is prevented, produce sporophytic buds.

FARMER and DIGBY (4) reported that in *Lastrea pseudo-mas* var. *polydactyla* Wills and *L. pseudo-mas* var. *polydactyla* Dadds fusions of vegetative nuclei replace gametic union, the result being a diploid cell or group of cells which develop into an embryo. No nuclear fusions occur in gametophytes of *L. pseudo-mas* var. *cristata apospora*, which is both aposporous and apogamous.

YAMANOUCHI (12) believed he could trace the development of the embryo of *Nephrodium molle* from a single superficial cell located on the ventral side of the prothallium close behind the apical notch. These initials could be detected within 5 weeks after the prothallia reached the two- or three-celled stage. Embryos are likewise formed sexually. HEILBRONN (6) added *Lastrea filix-mas* var. *grandiceps* Woll. to the list of apogamous forms.

STEIL (9) found that in the apogamous species, *Nephrodium hirtipes*, the last premeiotic divisions are incomplete, restitution nuclei with the doubled number of chromosomes resulting. Instead of the usual 16 spore mother cells and 64 spores, 8 and 32, respectively, are formed. STEIL believed this behavior to be indicative of hybrid origin. He found no nuclear migrations in the gametophyte.

STEIL (10) has given a generalized description of the development of embryos of several apogamous species, based on his own and others' observations. The position of an embryo varies from species to species, or even within a species; its location is usually ventral and back of the apical notch, but may be directly in the notch, on a process developed from the notch or on the wings of the prothallium. Generally the prothallial cushion has not developed when the apogamous embryo appears. Tracheids appear in a light green strip of the cushion before the gametophyte has reached full size, and precede the initiation of an embryo in

[1] This work was done in the Barker Cryptogamic Laboratory of the University of Manchester under the direction of Professor W. H. LANG. Appreciation is extended to Professor LANG, DR. IRENE MANTON, and Professor C. E. ALLEN for their interest and constructive criticism. Appreciation is expressed to the National Research Council for its support of this work and that on *Doodia caudata*.

some species. The embryo is usually surrounded by hairs consisting of single rows of cells; in at least two species scales are also present. The apical cell of the leaf differentiates first, next that of the root, and finally that of the stem. No foot is present. The root may outstrip the leaf in development. Secondary prothallia, provided they possess an apical notch, commonly give rise to embryos.

In sporangia of *D. remota* and *D. paleacea* var. *cristata*, Döpp (2) distinguished three types of behavior of spore mother cells which lead to varying degrees of fertility. Progeny of a mating between sexual *Dryopteris filix-mas* and apogamous *D. paleacea* var. *cristata* resemble the apogamous parent in possessing three types of spore mother cells and in producing apogamous gametophytes. The various sexual forms of the *D. filix-mas* complex he considered to have come from *D. filix-mas* proper; the apogamous varieties, from matings of *D. paleacea* or derived hybrids with forms of *D. filix-mas*. *D. remota*, reported to be apogamous by Fischer (5), is a hybrid between *D. paleacea* and *D. spinulosa*. Manton (8) has pointed out that *D. paleacea* Moore is synonymous with *D. pseudo-mas* Woll. Studies of prothallia of *Lastrea pseudo-mas* var. *polydactyla* Dadds and *L. pseudo-mas* var. *polydactyla* Wills led Döpp to the conclusion that nuclear migrations and fusion therein are obtained only after certain procedures in fixation.

Material and methods

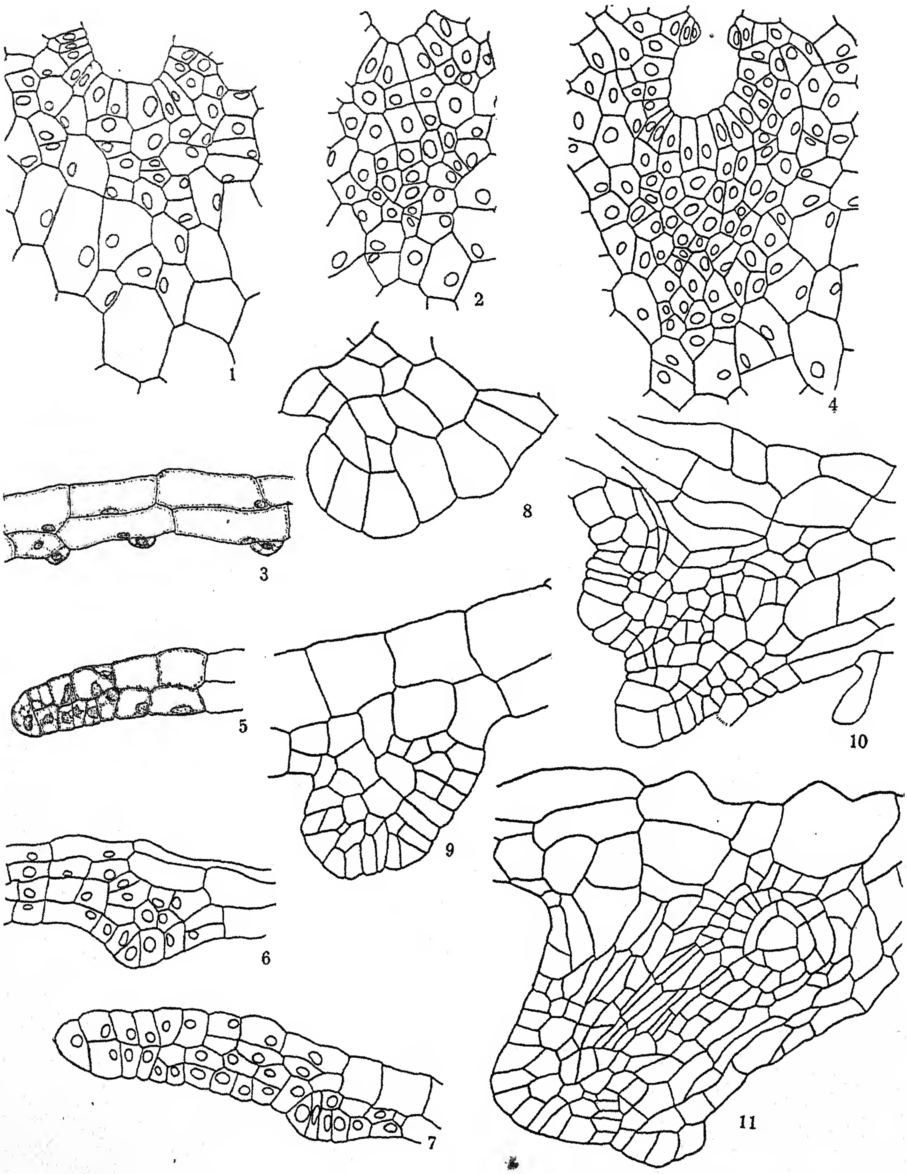
Prothallia of *Dryopteris pseudo-mas* (Woll.), *D. pseudo-mas* var. *polydactyla* Wills, *D. psuedo-mas* var. *polydactyla* Dadds, *D. pseudo-mas* var. *cristata* Cropper, *D. hirtipes* (Bl.) O. Ktze., and *D. mollis* Underwood were studied both in sectioned material and *in toto*. Dilutions of Karpechenko's, Merton 2 BE, and chrom-acetic solutions were used as killing agents. Whole prothallia were mounted in glycerin jelly and balsam. Prothallia bearing embryos and young sporophytes were cleared in Javelle water and stained with decolorized ammoniacal fuchsin. This material was used in the study of the vascular system.

All forms were present at the experimental gardens of the University of Manchester. The *polydactyla* varieties are of the same stock as those used by Lang, Farmer and Digby, Manton, and Döpp in their researches. Miss Manton kindly made available the triploid race of *Dryopteris pseudo-mas*.

Observations

Most of the study was devoted to *Dryopteris pseudo-mas*. Both irregular and heart-shaped prothallia are present. The former are without apical notches and do not form embryos but produce antheridia; the latter bear both antheridia and embryos (fig. 14). Several cases of doubtfully incompletely formed archegonia on heart-shaped prothallia were observed.

Young prothallia were studied to ascertain whether nuclear migrations and fusions play a role in the origin of the embryos. In no cases were nuclei found passing through pores in the walls or fusing with other nuclei. Figures 1 and 2 illustrate the cells on the ventral side behind the apical notch of young prothallia at two stages of development prior to initiation of embryos. In the central portion of the prothallium, just behind the developing cushion and anterior to the area in which antheridia and rhizoids may be present in profusion, apparently binucleate cells frequently occur. No empty cells adjoin them. Such a cell examined in section invariably possesses a small projection in which one nucleus



FIGS. 1–11.—Figs. 1, 2, 4, surface views of lowermost layer of cells just behind notch of successively older prothallia; ×200. Fig. 3, vertical longisection of young cushion region of prothallium, showing rhizoid initials; ×150. Fig. 5, vertical longisection through notch region; ×150. Fig. 6, same of embryo arising from cushion four cells thick; ×200. Fig. 7, same of embryo arising from cushion which apparently was two cells thick at time of origin and became four cells thick later; ×200. Fig. 8, lateral surface view of young embryo; ×300. Fig. 9, vertical longisection of embryo arising from cushion two cells thick. Apical cells of stem and leaf present; ×200. Fig. 10, same of young embryo at time leaf outstrips stem in growth; upper two cell layers of cushion little affected; ×200. Fig. 11, same (slightly oblique) of stem of young embryo; apical cell of first root present; ×200.

lies. Darker staining discloses the presence of a wall setting off the projection as a separate cell from the larger upper cell. The small cells so formed are rhizoid initials (fig. 3) and are not found in the area in which an embryo is to arise although they are located immediately behind it. Corresponding hair initials from marginal or superficial cells have similar aspects. There is no evidence of a causal relationship between such cells and the origin of an apogamously formed embryo.

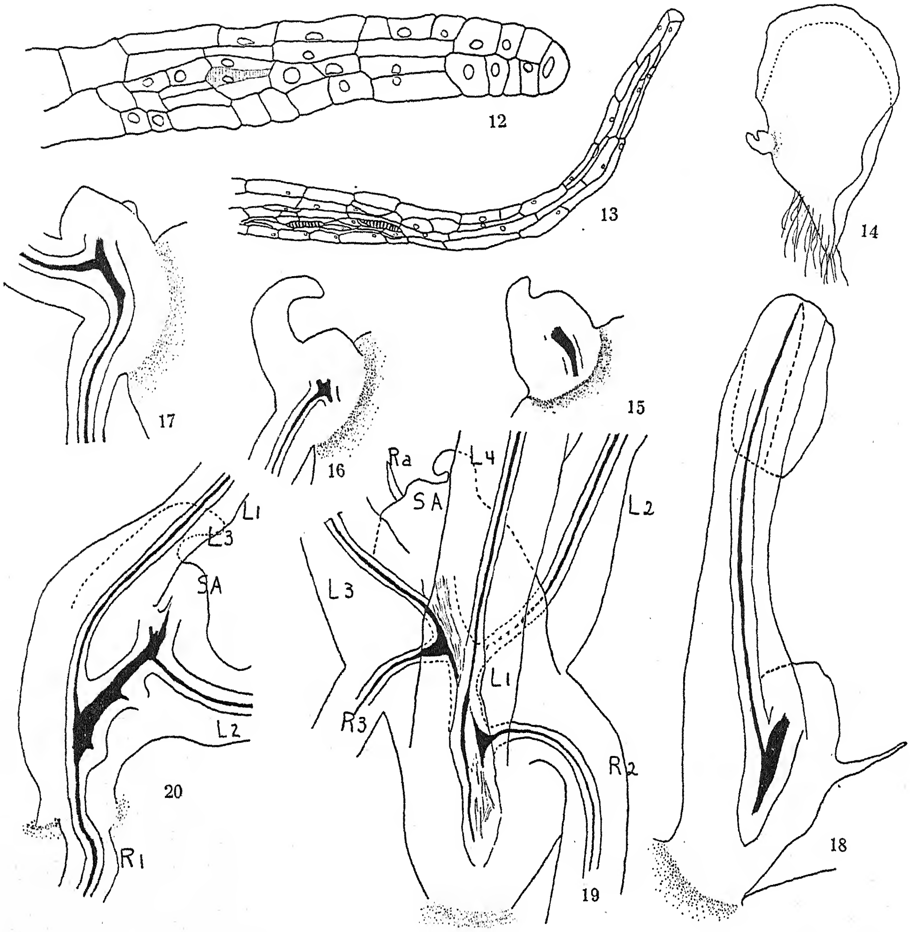
A young heart-shaped prothallium consists of a flat plate one cell in thickness, which is added to by divisions of the cells in the apical notch. When a prothallium reaches a length of approximately 2 mm. and a corresponding width, the cushion becomes two cells thick by division of the cells at a short distance behind the notch. On the ventral side of the cushion of a prothallium is a fairly distinct group of cells (fig. 4) somewhat smaller in size and richer in protoplasm than the adjoining ones. Vertical longitudinal sections of a prothallium at this stage show that the cells in this region on the ventral side are somewhat smaller than those on the dorsal (fig. 5). At least one division occurs in the cells of each layer, the cushion becoming four cells thick. Meanwhile the notch remains active, so that anterior to this thicker region there is a portion of cushion in formation which is as yet two cells thick and in which the cells are somewhat larger. In sections the first indications of apogamous embryo formation observed are cell divisions in layer 3 (the layer next to the lowermost layer in a cushion four cells thick), the resulting cells distend layer 4 (the lowermost layer). Soon the cells of layer 4 divide likewise, in pace with the increase of interior tissue, the planes of the divisions being vertical while most of the interior divisions are in horizontal planes.

Within the layer formed by the divisions of the cells of layer 4 an apical cell is constituted. Figure 6 shows the upper two cell layers of the cushion as yet little affected, the irregular group of cells derived from layer 3, and the appearance of an apical cell in layer 4. The apex diverges slightly forward and downward at about the same angle as lies between the older embryo and the prothallium. If the apical cell does not differentiate and the cushion cells elongate, the interior cells derived from layer 3 elongate likewise and some of them may differentiate as tracheids (fig. 12). Later new prothallial growing points may be constituted along the anterior or inner lateral (next the notch) margin of the wing. An embryo may arise in tissues derived from these new growing points. Embryos have not been observed to originate in the notch tissue directly.

In some cases the embryo arises from a cushion two cells in thickness (fig. 9). Figure 8 illustrates the formation of a small tonguelike bulge following cell divisions on the posterior faces of the apical cell of the newly constituted embryo. Behind such an apex and away from the apical notch of the prothallium a second apical cell arises in one of the segments derived from the first (fig. 9). The original apical cell of the protuberance, the young embryo, is the apical cell of the stem, while the one back along the protuberance is that of the primary leaf. Hairs are now present, growing out from the prothallial cells of layer 4 in the vicinity of the young embryo.

The stem maintains for a time the dominant position shown by the position of the embryo; however, the leaf soon outstrips it in development (fig. 10) and at times slightly displaces the stem to-

ward the notch of the prothallium. By the time the primary leaf has grown well forward and started to bend around the stem apex, considerable stem tissue has resulted from the activity of the apical cell of the stem. The interior cells of this young stem are elongated along the axis lying behind the stem apex. At the base of this young stele, cells derived from layer 3 of the prothallium still possess the power of division. Within the limits of this tissue one cell enlarges considerably more than the surrounding cells and then divides successively in several tangential planes, so that a noticeable sheath is present. This is the apical cell of the primary root (fig. 11). During or immediately before the differentiation of the



FIGS. 12–20.—Fig. 12, vertical longisection of cushion in which internal tissue developed from layer 3 but apical cell failed to appear in layer 4; ×200. Fig. 13, vertical longisection of elongated cushion containing tracheids derived from layer 3; ×100. Fig. 14, young embryo attached to heart-shaped prothallium whose two wings are folded back; ×20. Figs. 15–20, sketches of embryos cleared and stained so that xylem and endodermis are visible. L1, L2, L3, and L4 are first, second, third, and fourth leaves, respectively. R1, R2, and R3 are first, second, and third roots. SA, stem apex; Ra, ramentum. Figs. 15 and 16, ×40; figs. 17–20, ×50.

root apex, cells of layers 1 and 2 occasionally divide, no great amount of tissue resulting. In the case of one embryo arising from a cushion two cells in thickness, the earliest root departed from the stem at some distance from the insertion of the stem on the prothallium, the root associated with the first leaf being absent (fig. 19). The effect is that of an embryo seated on a stalk; however, the anatomy of the stalk is stemlike.

At about the time of root initiation, the cells of the cushion lying between the apical notch of the prothallium and the point of embryo insertion elongate, the elongation involving generally the meristematic cells at the base of the notch and less frequently those bordering its sides. The result is a general broadening of the notch, so that the wings lie farther apart, and at the same time the wings turn upward. A group of cells from the base of the notch or from the margin of the wing adjoining the notch may push out to form a "middle lobe." There are cases in which a second embryo forms on the portion of the cushion ahead of the first successfully established embryo and, peculiarly, some in which the second embryo lacks the apical cell of a stem derived from the development of layer 4. In this latter case the internal cells (derived from layer 3) of the second embryo, the only evidence of its presence, become involved in cushion changes, and after elongation some become tracheids. Elongation of the cells of the cushion, as well as occasional divisions of the cells of the notch meristem before it loses its embryonic qualities, causes the embryo to lie farther back along the cushion. Once the embryo is well established and elongation of the cushion has taken place, the prothallium concerned has no further development.

An embryo may abort as a whole, or the primary leaf occasionally and the primary root more frequently may abort separately. The earliest abortion occurs at the failure of the first apical cell to appear. In this event the internal cells elongate with the cushion, and some become tracheids, the only evidence of an aborted embryo being the presence of the tracheids in a somewhat thickened cushion (fig. 13). Even though a small protuberance may have been formed through the activity of the first apex, the embryo, particularly as the culture grows older, may fail to continue actively meristematic. Its cells grow larger and become more vacuolate. The differentiation of cells from layer 3 provides tracheids found in elongated cushions more thickened than in the first type of abortion mentioned. Prothallia with such cushions give rise to secondary prothallia, whose origin lies in the meristematic activity of cells just ahead of the elongated cells bordering the wing margin adjoining the prothallial notch, or of marginal cells along the anterior edges of the wing. Secondary prothallia give rise to embryos which may develop or abort. Occasionally an embryo arises early behind a secondary growing point on the inner margin of a prothallial wing. Such an embryo is apparently located on the wing near the notch, since the other wing of the secondary prothallium is as yet small or nonexistent. One arising still earlier and nearer the notch would be classified as arising in the notch, of course; this location has not been observed.

The stele differentiates and matures acropetally in stem, root, and leaf, development of the endodermis keeping pace with that of the protoxylem. In a young embryo bearing one leaf (fig. 15) it is difficult to determine whether the main axis is from root to stem apex or from root to

leaf apex. The stem apex is always present, however; the stele differentiates directly toward it (fig. 16). When the leaf trace has passed out into the leaf base, the vascular system of the stem has extended beyond the insertion of the leaf trace (fig. 17). Older embryos demonstrate that even the first leaf is lateral, since the stem grows directly forward while the first and later leaves diverge from it at about the same angles (fig. 19). When the first root is not present the line of tracheids ceases sharply at the point where the root apex would arise (fig. 18); when it is present the stele of the root is directly continuous with a portion of the stem stele (fig. 17). Occasional tracheids appear in the cushion immediately ahead of the embryo; these are differentiated cells derived from layer 3 of the young prothallium (fig. 20).

Each leaf diverges at an angle of about 100° from the previous leaf (phyllotaxy was not determined), and each is associated with a root which arises endogenously from just below its base. Exceptions to this association are the failure of the first root to appear (fig. 19), or, in the event that this root is present, the frequent abortion of the second root (fig. 20). Primordia of all leaves originate as small moundlike outgrowths among occasional young ramenta on the conical stem tip (fig. 19).

The remaining forms studied have all been treated rather extensively by other workers, so that detailed description is hardly necessary. In general, events seemed to move more or less in the same manner as in *Dryopteris pseudo-mas*. However, certain atypical developments have been thought worthy of listing.

In *Dryopteris pseudo-mas* var. *polydactyla* Wills a relatively massive primary root often develops precociously, even earlier than the primary leaf. In at least one case the primordium of the primary leaf had not appeared, but a well-defined axis between stem and root apex was present.

The tendency toward the development of a strand of tracheids between the embryo and the prothallial notch in *Dryopteris pseudo-mas* var. *polydactyla* Dadds is pronounced. Since at the time of embryo initiation more cells of layer 3 than in triploid *D. pseudo-mas* are involved in the formation of internal tissue, this tissue can occupy a longer portion of the cushion, there being, therefore, greater opportunity for the root to arise at some distance behind the stem or for the tracheidal strand to differentiate forward of the embryo and toward the notch. One compound embryo was observed which consisted of two stems and their associated leaves all related to one primary root.

The prothallium of apogamous and aposporous *Dryopteris pseudo-mas* var. *cristata* Cropper differs now and then from the species in the extension of the internal tissue for a short distance behind the insertion of the embryo. The appearance of the primary root is generally delayed; the first leaf, too, may arise late from a stem which is a cylindrical process terminating in a conical tip. Abortion is frequent. Elongation of the cushion region and segregation of possible growing points along the margin of the prothallium—whether anterior or bordering the notch—lead to formation, by regeneration, of secondary prothallia which are characteristically asymmetrical and produce embryos which chiefly abort. Abortion is generally of the type in which an apical cell does not differentiate in the lowermost layer and in which cells of the interior tissue and of the other layers of the cushion elongate. Middle lobes derived from tissue at the base of the notch occur frequently in the prothallia of this variety.

In *D. hirtipes* the functional association of the tissue derived from layer 3 and of the apex derived from layer 4 is not so close as in the forms previously mentioned. The interior tissue is not so limited and may be formed seemingly independently of any development from layer 4. Among the posterior cells derived from layer 3 the apical cell of the root appears; behind it tracheids differentiate from the stele of the stem. The root may grow out of the cushion tissue, or the root apex may remain in its initial situation. The tendency toward development of tracheids, or at least extension of the endodermis toward the notch region, is common. Two adjacent embryo initials in line with the prothallial notch have been seen. In the course of development they may become related to one primary root. The stem of the posterior embryo is usually abortive and possesses only a few tracheids. Its leaf appears as a lateral outgrowth along the axis between the root apex and the stem of the anterior embryo. In one case the axis of development ran from root apex to prothallial notch and a leaf developed laterally; no stem apex was discernible.

Prothallia of *D. mollis* having only a capillary source of water and of considerable age had peculiar localized thickenings behind the notch region. Sections showed them to be sexually formed embryos which had not erupted from the distended venters of archegonia. No apogamous embryos were observed; the amount of material, however, was limited.

Discussion

De Bary (1) noted that the upper cell layers of the prothallium in *Pteris cretica* are not involved in the apogamously produced embryo which arises from the lower cell layers. These observations are confirmed in this study and are expanded by observations on the divisions of function between the lower two cell layers (layers 3 and 4) of the prothallial cushion as outlined under the treatment of *Dryopteris pseudo-mas*.

The first apical cell is interpreted as that of the stem and the second as that of the primary leaf. This interpretation naturally culminates in the conclusion that the primary leaf of the apogamously produced embryo is lateral in attachment, not first to appear and terminal in attachment as previous workers have indicated. The order of appearance is borne out by comparison of a close series of young embryos (figs. 8, 9, 10, 15, 16). Collateral evidence is the fact that the main axis of differentiation is from the base of the primary root to the stem apex, while the traces leading into the primary leaf and into all later leaves are lateral and not so large as the stele of the stem.

The trace of the primary root originates at the base and toward one side of the vascular system of the stem. Each later root arises next the stele and proximal to the leaf with which it is associated, so that the two traces (of root and leaf) plus the portion of the stele of the stem lying between them form a distinct U. This association of leaf and root is the "phyllorhize" of various investigators, but the present investigation does not support the ultimate conception of the phyllorhize theory that the stem is a collection of traces connecting roots and leaves and that the stem apex arises anew at the base of each new leaf. The results indicate that the stem is a permanent structure, that its apex is present from the beginning, and that the differentiation of tissues within the stem is abreast, ahead many times, of the youngest leaf in whose base a trace is differentiating. Those classes of embryos, al-

though of infrequent occurrence, in which the primary leaf appears late, aborts, or never forms at all so that the stem is conspicuous and those in which the primary leaf appears as the lateral outgrowth of an axis running from the primary root to the notch of the prothallium, the stem having aborted, bear out the contention that the leaf is a lateral appendage.

Several differences between the obligate and induced types of apogamy seem clear. In the obligate type generally a single embryo appears early—at about the time when non-apogamous prothallia are producing archegonia—and arises from a definite area of the prothallial cushion; whereas sporophytic buds of the induced apogamous type, frequently numerous, generally arise late in the development of comparatively larger, more massive, highly modified prothallia, and from no preordained portion of the prothallia. The relative place of tracheid differentiation within the prothallia is characteristic for each type. If the cytological behavior of *Nephrodium hirtipes* or *Aspidium remotum*, described by STEIL (9) and DÖPP (2), respectively, should be found in all the obligate types, then all portions of the life cycle have the same or very similar chromosome number as the result of restitution nuclei formed in the last premeiotic division. The true induced type of apogamy leads to haploid sporophytes produced directly from prothallia with the reduced number of chromosomes, sporogenesis having been normal (3).

The embryo of a sexually produced sporophyte differs from that of one produced normally in that a foot is present. An apogamously produced embryo, however, arises directly from the prothallium and remains connected therewith, no special absorptive structure being necessary. Here may lie an explanation of the involvement of the prothallium in sporophytic tendencies, such as tracheid formation in the cushion. It might be assumed that the changes in question in the cushion are expressions of slightly variable growth-regulatory phenomena. The distinctly sporophytic cells of the embryo and the distinctly prothallial cells, of course, are precisely alike genetically and vary cytologically only in details of cell organization.

Summary

1. The lower two cell layers of the four-layered portion of the prothallial cushion just behind the apical notch are concerned in the initiation of an apogamously produced embryo of *Dryopteris pseudo-mas*. Divisions occur first in the upper of the two ventral layers (layer 3). Before these divisions have ceased, cells of the lower ventral layer begin to divide. The upper two cell layers are not concerned in these changes, although later a few cell divisions may take place. Prothallia whose cushions are two cells in thickness may give rise to embryos, the lower layer behaving as layer 4.

2. The tissue formed from layer 3 gives rise to the root apex and to cells of the prothallial cushion which may become tracheids. Layer 4 gives rise to an apical cell; in a segment derived from it a second apical cell differentiates. The former is the apical cell of the stem; the latter that of the primary leaf.

3. Abortion of the entire embryo, when it occurs, is a consequence of the failure of the first (stem) apex to differentiate. Any particular organ may abort independently of the other organs.

4. Tracheids in the prothallial cushion which bears no embryo are evidences of the abortion of the whole embryo, with

the possible exception of the apical cell of the root, which—except in sectional view—would be indistinguishable. Tracheids lying in the cushion ahead of a developing embryo are evidences that the changes in layer 3 in preparation for embryo initiation involved more of the cushion than the changes in layer 4. Such tracheids arise along an axis between the prothallial notch and the base of the stem.

5. The four forms of the *Dryopteris pseudo-mas* complex and *D. hirtipes* show a highly similar method of apogamous embryogeny.

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LITERATURE CITED

1. DE BARY, A., Über apogame Farne und die Erscheinung Apogamie im Allgemeinen. Bot. Zeitung **36**:449–487. 1878.
2. DÖPP, W., Cytologische und genetische Untersuchungen innerhalb der Gattung *Dryopteris*. Planta **29**:481–533. 1939.
3. DUNCAN, R. E., Apogamy in *Doodia caudata*. Amer. Jour. Bot. **28**:921–931. 1941.
4. FARMER, J. B., and DIGBY, L., Studies in apospory and apogamy in ferns. Ann. Bot. **21**:161–201. 1907.
5. FISCHER, H., Apogamie bei Farnsbastarden. Ber. deutsch. Bot. Gesell. **37**:286–292. 1919.
6. HEILBRONN, A., Apogamie, Bastardierung, und Erblichkeitsverhältnisse bei einigen Farnen. Flora **101**:1–42. 1910.
7. LANG, W. H., On apogamy and development of sporangia upon fern prothallia. Phil. Trans. Roy. Soc. London Ser. B. **190**:187–238. 1898.
8. MANTON, I., Cytology of the common male fern in Britain. Nature **144**:291–292. 1939.
9. STEIL, W. N., A study of apogamy in *Nephrodium hirtipes*. Ann. Bot. **33**:109–132. 1919.
10. ———, Apogamy, apospory, and parthenogenesis in the pteridophytes. Bot. Rev. **5**:433–453. 1939.
11. WOLLASTON, G. B., Forms of *Lastrea Filix-mas*. Phytologist **6**:415. 1863.
12. YAMANOUCHI, S., Apogamy in *Nephrodium*. BOT. GAZ. **45**:289–318. 1908.

ANATOMICAL AND CHEMICAL ASPECTS OF ABSCISSION OF FRUITS OF THE APPLE[1]

MONROE McCOWN

Introduction

Beginning immediately after the petals fall and extending over a period of 5 or 6 weeks, abscission of many of the immature fruits of the apple normally occurs. Abscission of the developing fruits continues, but further shedding of any considerable number of uninjured fruits within a limited period usually does not occur until the approach of fruit maturity. Information on the anatomical development of the tissues involved and the chemical changes in these tissues preceding and during abscission is limited. In this investigation the processes involved in the formation of the absciss layer were studied and are herein described.

HEINICKE (10) concluded that the separation results from the activity of cells near the base of the pedicel. NAMIKAWA (16) reported cell division in the "abscission zone" just prior to the drop of apple fruits. He attributed cell separation to the combined effects of increased turgor and the dissolution of the middle lamella and a part of the secondary cell wall. FEHER (8) also reported that cell division occurs just preceding the separation. McDANIELS (14) noted the occurrence of cell division in connection with the formation of the "abscission layer" of flowers and young fruits but observed no proliferation of cells preceding the dropping of mature fruits.

METHODS.—The studies were initiated in 1936 and extended over a period of five seasons. An orchard near West Lafayette, Indiana, was the source of the material. The trees, 13 years of age when the studies were begun, produced moderate to heavy crops except in 1938, when a freeze in mid-May destroyed a major portion of the fruit. The collections were made at frequent intervals, from approximately 1 week preceding flower opening until after commercial harvest.

While the varieties Rome Beauty, McIntosh, Grimes Golden, Golden Delicious, Delicious, and Fameuse were included in the microchemical studies and general observations of fresh material, permanent slides were prepared only from samples of McIntosh and Rome Beauty. These samples consisted of small portions of stems embracing parts of both the pedicels and the peduncles of flowers and fruits. The term "peduncle" is applied to that portion of green stem at the tip of the cluster base from which issue the pedicels of flowers or fruits of the apple (13).

The material was left in Rawlin's formalin-acetic acid-alcohol killing solution until needed. Older material was softened in hydrofluoric acid for 5–8 weeks. Dehydration was in ethyl alcohol. The short celloidin schedule was followed in preparing the samples for sectioning. The sections, varying 8–15μ in thickness, were stained in Ehrlich's haematoxylin and safranin and mounted in Canada balsam.

For the microchemical studies and supplementary observations, fresh spurs with pedicels attached were collected and placed in water to prevent drying. All fresh material was studied within a few hours. Freehand sections were cut with a razor blade from the fresh stem portions, except that some fresh flower pedicels were balled in paraffin and sectioned on a microtome. A microscope with disk polarizer and analyzer attachments was utilized in the optical studies.

[1] Journal Paper no. 105, Purdue University Agricultural Experiment Station.

The standard tests were followed in the microchemical studies, as follows:

CELLULOSE.—Hydro-cellulose reaction; cupra-ammonia; polarized light.
PECTIC COMPOUNDS.—Polarized light; ruthenium red; solubility.
PECTIN.—Water-soluble.
PECTIC ACID.—Two per cent KOH.
CALCIUM PECTATE.—Three per cent ammonium oxalate; 2 per cent H_2SO_4 or 2 per cent HCl followed by 2 per cent KOH.
PECTOSE.—Heated for 30–60 minutes in 2 per cent HCl followed by dilute alkali.
LIGNIN.—Phloroglucin-HCl; polarized light (15, 19, 7, 18).

Throughout this paper, an effort has been made to conform to the terms suggested by KERR and BAILEY (12) and accepted also by ANDERSON (1): Middle lamella—the amorphous, isotropic material, largely (if not entirely) pectic compounds first deposited by the cytoplasm. Primary wall—the first anisotropic layer of the wall, composed largely of cellulose and pectic materials. Secondary wall—the additional wall layers formed by the cell.

Investigation

ANATOMY OF PEDICEL

IMMATURE PEDICELS.—At the pink stage of flower development (when the first collections were made), the pedicel was relatively soft. The cells of the various tissues were immature and mostly parenchymatous. In the pericycle some cells were evidently developing as fibers, others as stone cells. A very small amount of secondary xylem and phloem was evident.

Constriction of the base of the pedicel and apex of the peduncle was evident in immature stems (fig. 1). In this constricted zone the cells of all tissues, except those of the pith, were smaller than adjacent ones. This zone was usually 20–30 cells in width in the cortical tissue, less wide in xylem and phloem, and apparently constituted the so-called "abscission zone" of NAMIKAWA (16). Since the abscission process is not limited to this zone, however, it seems more appropriate to refer to it as a constriction zone, and it is so designated in this paper (fig. 1).

Enlargement and differentiation of cells in the pedicel proceeded slowly during the period of flowering and shedding of petals. Within a week after petal-fall, fibers and some stone cells in the pericycle, as well as isolated pith cells, exhibited wall thickening and evidence of lignification. Within 3 weeks after the petals fell, many pericyclic and pith cells were well-developed stone cells, and well-formed pericycle fibers were present. Walls of the cells in the constriction zone and in the cortex were much thickened.

No absciss layer is differentiated in pedicels of fruits that remain on the trees after the June drop is completed, and for the purpose of this discussion these surviving pedicels are considered as mature (fig. 2).

MATURE PEDICELS.—During development, cells of the pith, the secondary xylem, and the stone cells and the pericyclic fibers, together with the vessels in the primary xylem, were lignified. The secondary walls of the cortical cells, especially those in the outer and basal portions of this tissue in the pedicel, became much thickened as the result of deposition of cellulose; the thick walls of the small parenchymatous cells characterizing the constriction zone remained chiefly of cellulose.

In the pedicels of fruits of Rome Beauty, Golden Delicious, and Delicious at harvest time, the middle lamella was chiefly pectose, except in the cambial region, cortex, and in the constriction zone —where it was mostly a calcium salt of

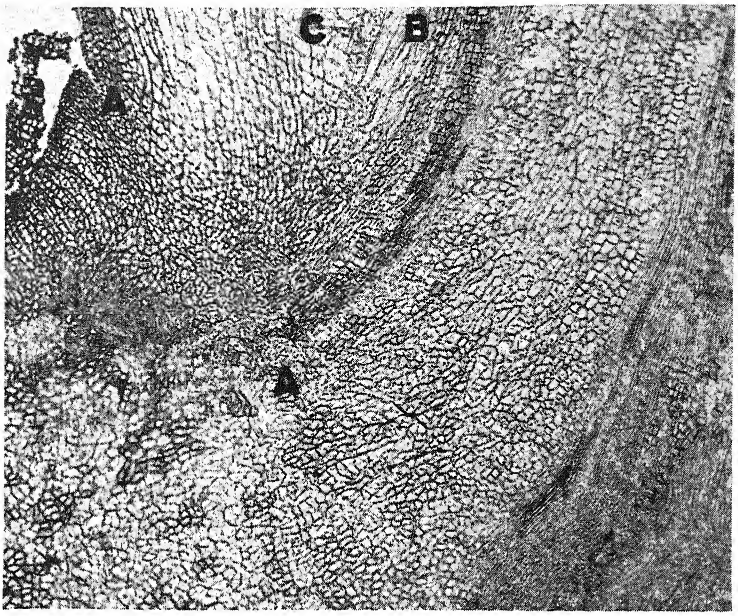

FIG. 1.—Constriction zone of small cells (A–A) evident in immature apple pedicel at juncture with peduncle. In pericycle, cells are being modified to fibers (B) and stone cells (C). McIntosh, 1 week after petal-fall.

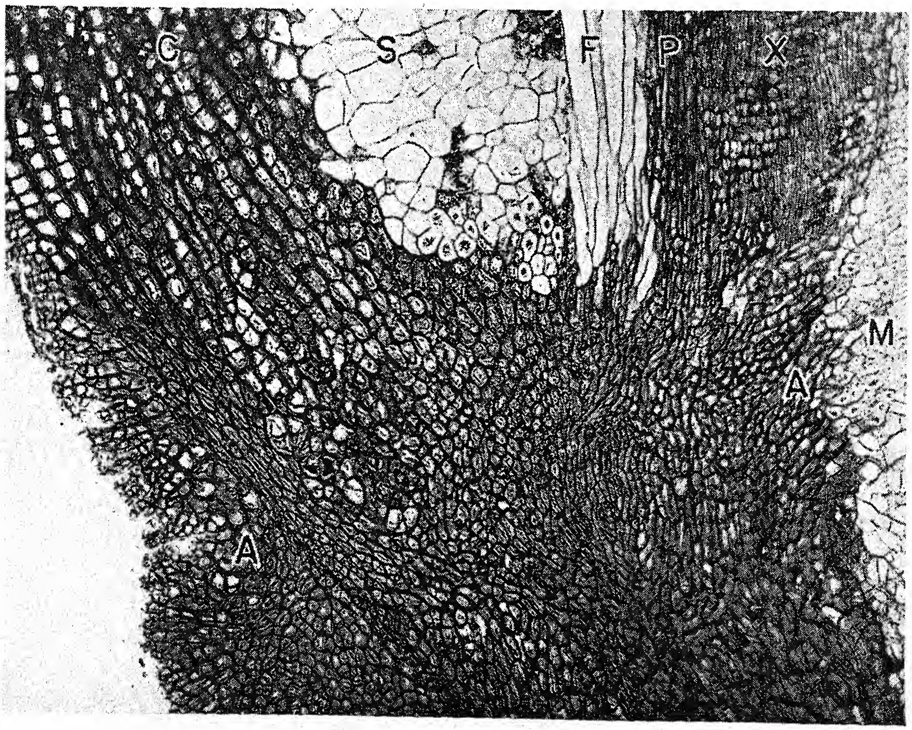

FIG. 2.—Longisection of mature pedicel. At end of June drop, vigorous pedicels appear similar to older one shown here. C, cortex; S, stone cells; F, fibers; P, phloem; X, xylem; M, pith; A–A, constriction zone. McIntosh, 15 weeks after petal-fall.

pectic acid. Before the natural process of abscission had reached an advanced stage, separation of the stone cells of the pith could be induced only by heating in weak HCl, indicating the presence of pectose. The walls of the stone cells of the pith were chiefly of cellulose. Extremely thin sections of these cells were tested with phloroglucin-HCl. Very thin lamellae, in close association with the middle lamella and primary wall, and lining the pits and cell lumen, were the only portions of the walls to show positive reaction to this test. In many stone cells of the pith the lignified lamella lining the cell lumen was so thin that it was barely detectable and in some cells was entirely lacking. When treated with iodine and 75 per cent sulphuric acid, the secondary cell wall became swollen and blue and then very slowly disintegrated, indicative of its cellulose nature.

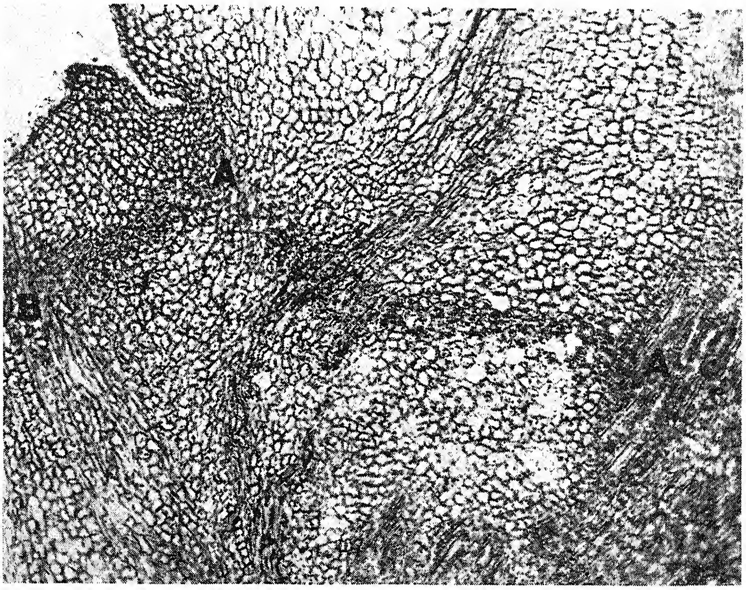

FIG. 3.—Longisection showing differentiation of absciss layer (*A–A*) preceding abscission of immature fruit. Contiguous layer (*A–B*) has formed across peduncle following abscission of terminal flower. Rome, 1 week after petal-fall.

Abscission process

IMMATURE PEDICELS.—Flowers and immature fruits abscised following differentiation of an absciss layer in the basal portion of the pedicel (figs. 3, 4). This layer resulted from cell division. In the abscising pedicels studied, the layer usually was differentiated within the limits of the constriction zone. In occasional pedicels, however, the layer appeared in the pedicel distal to the constriction zone. This fact has been reported by others (10, 14).

Cells in all tissues underwent division in the differentiation of the absciss layer, and the resulting layer was six to eight cells in width across the pedicel (figs. 3, 4). Absciss layers were not differentiated in pedicels that survived the June drop.

Following the fall of the terminal flower or immature fruit, the stump of the

peduncle abscised immediately above the most distal lateral flower remaining in the cluster. When all flowers or immature fruits in a cluster dropped, successive portions of the peduncle abscised following the differentiation of a series of two or more absciss layers, the entire peduncle being shed. These layers also were formed by cell division, and in origin and appearance they resembled the absciss layers involved in the shedding of the pedicels (fig. 3).

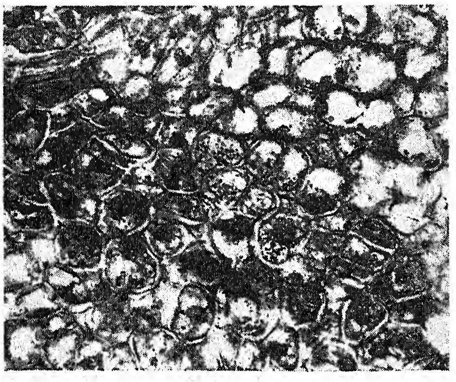

FIG. 4.—Small area of pith from center of section illustrated in fig. 3 showing cell division resulting in formation of absciss layer. Rome, 1 week after petal-fall.

A slight swelling of the middle lamella and primary wall was evident just preceding separation of the cells, which followed disintegration of the pectic compounds of the middle lamella and of the primary wall. There was little evidence of chemical modification of the secondary wall preceding separation. The vessels were ruptured, apparently as the result of mechanical force.

NAMIKAWA (16) also reported the disappearance of the pectic compounds from the walls of cells in the absciss layers of immature apple pedicels during abscission. SAMPSON (17) concluded that the separation of cells in the abscission of coleus leaves results from the dissolution of pectic compounds. APPLEMAN and CONRAD (2, 3), working with peach and tomato fruits, and CARRE (4, 5), CARRE and HORNE (6), and HALLER (9), studying the ripening of apples, concluded that softening of the fruits was due chiefly to the change of insoluble pectic materials to soluble forms. This seems identical with the course of events in the dissolution of the pectic compounds of the middle lamella and primary wall in the abscission of flowers and immature fruits of the apple.

Following the abscising of the pedicel or of the peduncle, cork was formed by a cambium which was initiated a few cells below the surface of the scar.

MATURE PEDICELS.—As stated earlier, pedicels of fruits that survived the June drop were mature, in so far as the mode of abscission is concerned. Division of cells and the consequent differentiation of a well-defined absciss layer were not observed in any mature pedicels. Rather, separation of the pedicel resulted from disintegration of the walls of pre-existing cells. The constriction zone did not determine the path of abscission.

Abscission was initiated independently in the pith and in the cortex of the pedicels studied (fig. 5). In cases where abscission was initiated first in the pith, its course was continued across the xylem, cambium, and phloem, meeting at some point in the bark tissues the line of separation which had arisen in the cortex. If abscission was initiated only in the pith, the separation progressed across the other tissues and was completed by the breaking of cortical and epidermal tissue. The weight of the fruit usually caused the final separation, chiefly by tearing the tissues of the outer portion of the cortex and the epidermis following parting of the inner tissues.

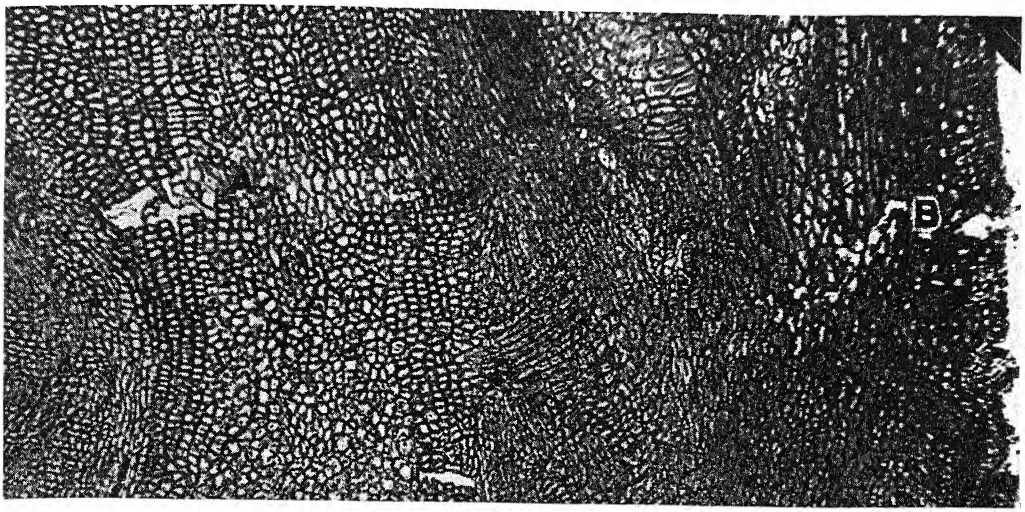

FIG. 5.—Longisection showing abscission of mature fruits initiating independently in pith (A) and cortex (B). Abscission may be initiated concurrently as illustrated here or begin first in either tissue. McIntosh, 15 weeks after petal-fall.

In some pedicels abscission was initiated first in the cortex, sometimes only in this tissue, and progressed toward the woody cylinder and pith. In which tissue abscission was first initiated, and whether the process was limited to the constriction zone, differed with varieties. The two extremes were in McIntosh and Golden Delicious.

In the mature pedicels of McIntosh, pith abscission usually preceded initiation of the process in the cortex. Separation usually occurred within the limits of the constriction zone. In the pedicels of Golden Delicious, cortical abscission preceded initiation of pith abscission as a rule. In most pedicels of Golden Delicious, abscission occurred in the pedicel distal to the constriction zone, and the line of separation ranged further from this zone as it crossed the pericycle and phloem (fig. 6). Limited observations indicated that the abscission of Grimes Golden pedicels resembled that of McIntosh in this respect. The sequence of pith and cortical abscission in most of the pedicels of Rome Beauty, Fameuse, and Delicious was the same as that in the Golden Delicious pedicels studied.

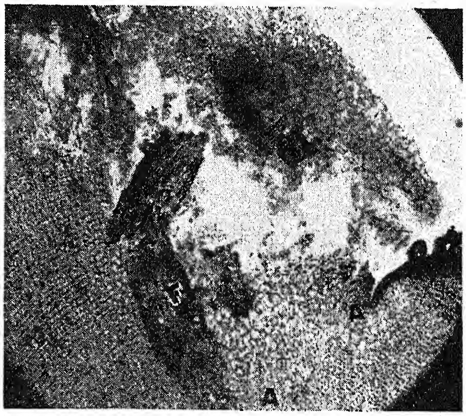

FIG. 6.—Longisection showing advanced stage of abscission in bark tissues of mature pedicel. Separation occurred at base, distal to constriction zone A–A, and course ranged upward as it crossed pericycle and phloem. Cells in softer tissues separated at middle lamella, while fibers (F) were ruptured. Golden Delicious, 22 weeks after petal-fall.

In the pith of mature pedicels, separation was preceded by more or less swelling and extension of the walls of a few

tiers of cells (fig. 7). Both the stone cells and any parenchyma located in this area became swollen and extended. During the process of swelling and extension of thick walls of the pith cells, the anisotropic quality was lost, indicating a physical change in the cellulose. HODGSON (11) noted this same change in thick-walled cells in abscising citrus leaves.

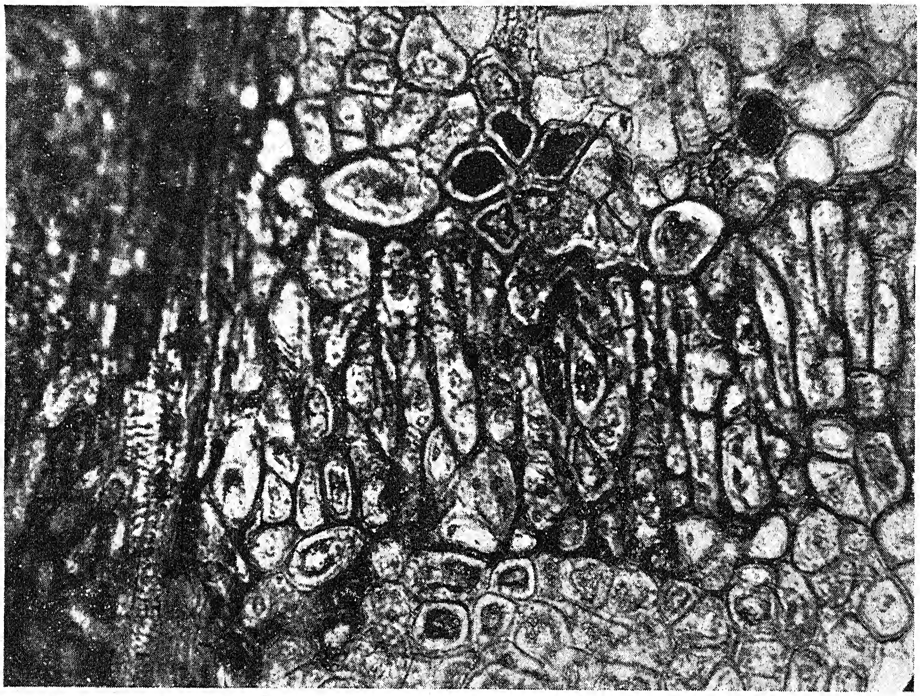

FIG. 7.—Longisection through base of mature pedicel showing cell separation in pith preceded by elongation and re-orientation of a few tiers of cells. Change in pectic compounds in middle lamella indicated by increased prominence of lamella. McIntosh, 15 weeks after petal-fall.

As abscission proceeded, the secondary cellulose wall gradually disintegrated. At the same time the character of the middle lamella was changed. The first indication of this change was an increase in its prominence resulting from swelling, and there was greater intensity of staining with ruthenium red (fig. 7). The pectic compounds of the middle lamella then dissolved, allowing separation of the cells (fig. 8). Just preceding the separation of the swollen cells, a positive test for pectic acid demonstrated a change from insoluble to soluble forms of pectic materials. Following separation, the cells collapsed and the remaining membranes of the disintegrating pith cells were made up of the lignified lamellae and a thin remnant of the secondary wall (fig. 8).

In tissues other than the pith, less disintegration of the cellulose of the secondary wall accompanied dissolution of the pectic compounds. Vessels and fibers in the path of abscission were ruptured by mechanical stress.

Following separation of the pedicel, the cells at the surface of the wound

dried and collapsed. Within 2 weeks the wound was well protected by cork.

Summary

1. A constriction zone, evident in the flower pedicels, persisted during the life of the apple fruits studied but did not function directly as an absciss layer or predetermine the course of abscission in pedicels of flowers or fruits.

2. The abscission of flowers and of immature fruits was preceded by differentiation of an absciss layer. Cell separation followed dissolution of the middle lamella.

3. Abscission of mature pedicels was initiated independently in the pith and cortex and not preceded by cell division.

4. The thick walls of the stone cells of the pith of mature pedicels were made up largely of cellulose lamellae. Separation of these cells was preceded by swelling and extension of the walls. During this process these cell walls lost their anisotropic quality, indicating a change in the nature of the cellulose.

5. In the course of cell separation in the pith, dissolution of the pectic compounds of the middle lamella and disintegration of the primary and much of the secondary cell walls occurred, leaving the lignified lamella and remnants of the secondary wall. In the separation of cells in tissues other than the pith, less disintegration of the secondary wall accompanied dissolution of the middle lamella and primary wall. Vessels and fibers in the path of separation were ruptured.

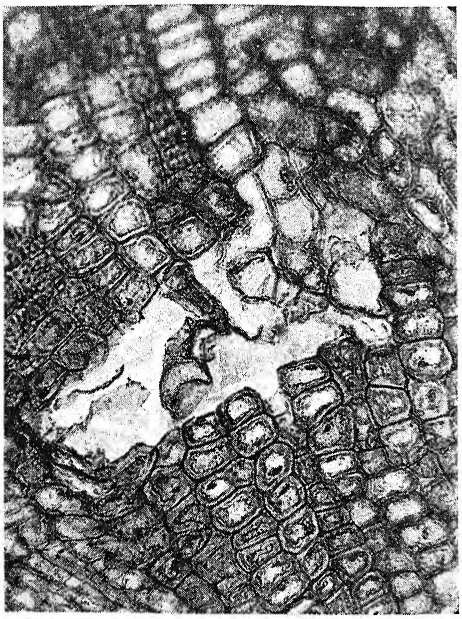

FIG. 8.—Enlargement of portion of pith area of section illustrated in fig. 5 showing dissolution of cell walls and advanced stage of pith abscission in mature pedicel. Remaining membranes of disintegrating cells, now supported by celloidin film, represent chiefly thin lignified lamellae and remnants of secondary walls. McIntosh, 15 weeks after petal-fall.

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LITERATURE CITED

1. ANDERSON, D. B., The structure of the walls of the higher plants. Bot. Rev. 1:52–76. 1935.
2. APPLEMAN, C. O., and CONRAD, C. M., Pectic constituents of peaches and their relation to softening of the fruit. Maryland Agr. Exp. Sta. Bull. 283. 1926.
3. ———, The pectic constituents of tomatoes and their relation to the canned product. Maryland Agr. Exp. Sta. Bull. 291. 1927.
4. CARRE, M. H., An investigation of the changes which occur in the pectic constituents of stored fruit. Biochem. Jour. 16:704–712. 1922.

5. CARRE, M. H., Chemical studies in the physiology of apples. IV. Investigation of the pectic constituents of apples. Ann. Bot. **39**:811–839. 1925.

6. CARRE, M. H., and HORNE, A. S., An investigation of the behavior of pectic materials in apples and other plant tissues. Ann. Bot. **41**:193–237. 1927.

7. CHAMOT, E. M., and MASON, C. W., Handbook of chemical microscopy. Wiley and Sons. 1930.

8. FEHER, DANIEL, Untersuchungen über den Abfall die Fruchte einiger Holzpflanzen. Ber. deutsch. Bot. Ges. **43**:52–61. 1925.

9. HALLER, M. H., Changes in the pectic constituents of apples in relation to softening. Jour. Agr. Res. **39**:739–746. 1929.

10. HEINICKE, A. J., Concerning the shedding of flowers and fruits and other abscission phenomena in apples and pears. Proc. Amer. Soc. Hort. Sci. **16**:76–83. 1919.

11. HODGSON, R. W., An account of the mode of foliar abscission in citrus. Univ. Calif. Pub. (Bot.) **6**:417–428. 1918.

12. KERR, T., and BAILEY, I. W., Structure, optical properties, and chemical composition of the so-called middle lamella. Jour. Arnold Arb. **15**: 327–349. 1934.

13. KRAUS, E. J., and RALSTON, G. S., The pollination of the pomaceous fruits. III. Gross vascular anatomy of the apple. Oregon Agr. Exp. Sta. Bull. 138. 1916.

14. MCDANIELS, L. H., Some anatomical aspects of apple flower and fruit abscission. Proc. Amer. Soc. Hort. Sci. **34**:122–129. 1937.

15. MOLISCH, H., Mikrochemie der Pflanze. Jena. 1923.

16. NAMIKAWA, ISAWA, Über die vorzeitige Abstossung der jenigeie Fruchte von *Malus communis*. Jour. Coll. Agr. Hokkaido Imp. Univ. Sapporo **11**:1–21. 1922.

17. SAMPSON, H. C., Chemical changes accompanying abscission in *Coleus blumei*. BOT. GAZ. **66**: 32–53. 1918.

18. ———, Laboratory manual in microchemistry. Ohio State University.

19. TUNMANN, O., Pflanzenmikrochemie. Berlin. 1913.

CYTOLOGICAL OBSERVATIONS IN CITRUS: III. MEGASPOROGENESIS, FERTILIZATION, AND POLYEMBRYONY

OSWALDO BACCHI

Introduction

An extensive program of investigation of the genus *Citrus* has recently been organized by several divisions of the Instituto Agronômico, its Limeira Citrus Experiment Station, and the Agricultural College at Piracicaba—all located in the State of São Paulo. The present paper discusses the megasporogenesis and embryology of *Citrus;* and a new type of polyembryony, not yet described for this genus, is presented.

STRASBURGER (10) was the first to make detailed cytological investigations in this genus. He not only described microsporogenesis and megasporogenesis, fertilization and endosperm formation, but he also explained the formation of adventitious embryos ("sporophytic polyembryony"), first discovered in 1719 by LEEUWENHOEK. The conclusions of OSAWA (6), who investigated *Poncirus trifoliata* and the varieties Unshiu (*C. nobilis* Lour.) and Washington Navel (*C. sinensis* Osbeck), agreed with STRASBURGER'S findings as to the origin of the adventitious embryos, which were shown to develop from cells of the nucellus surrounding the megagametophyte. FROST (4, 5) proposed the hypothesis of cleavage polyembryony to explain the production of two hybrid seedlings from a single seed, as among 1200 hybrids he found ten such pairs which gave rise to apparently identical twins.

Similar cases were found by TRAUB and ROBINSON (14); from 782 seeds from a cross between lemon and trifoliata orange, sixteen produced two hybrid seedlings, one three, and another four hybrids. They state that twin hybrids were also found by SWINGLE in 1909.

Various aspects of polyembryony in *Citrus* and related genera (*Poncirus* and *Fortunella*) have also been investigated (9, 12, 15–17, 13), and a revision of the subject has been presented by J. M. WEBBER (18). So far as known, only two types of polyembryony have been reported for this group of genera, nucellar and cleavage (4, 5), the latter caused by "fission of the generative (sexually produced) embryo."

MATERIAL AND METHODS.—Material was collected at the Citrus Experiment Station at Limeira of the Instituto Agronômico, from representative trees of two species, Grapefruit Foster (*Citrus paradisi* Macf.) and Sour Orange (*C. aurantium* L.).

For the study of megasporogenesis, ovaries were collected from buds at different stages of development and also from recently opened flowers. For other observations the ovaries were collected at intervals from flowers emasculated before anthesis and artificially pollinated 4 days later.

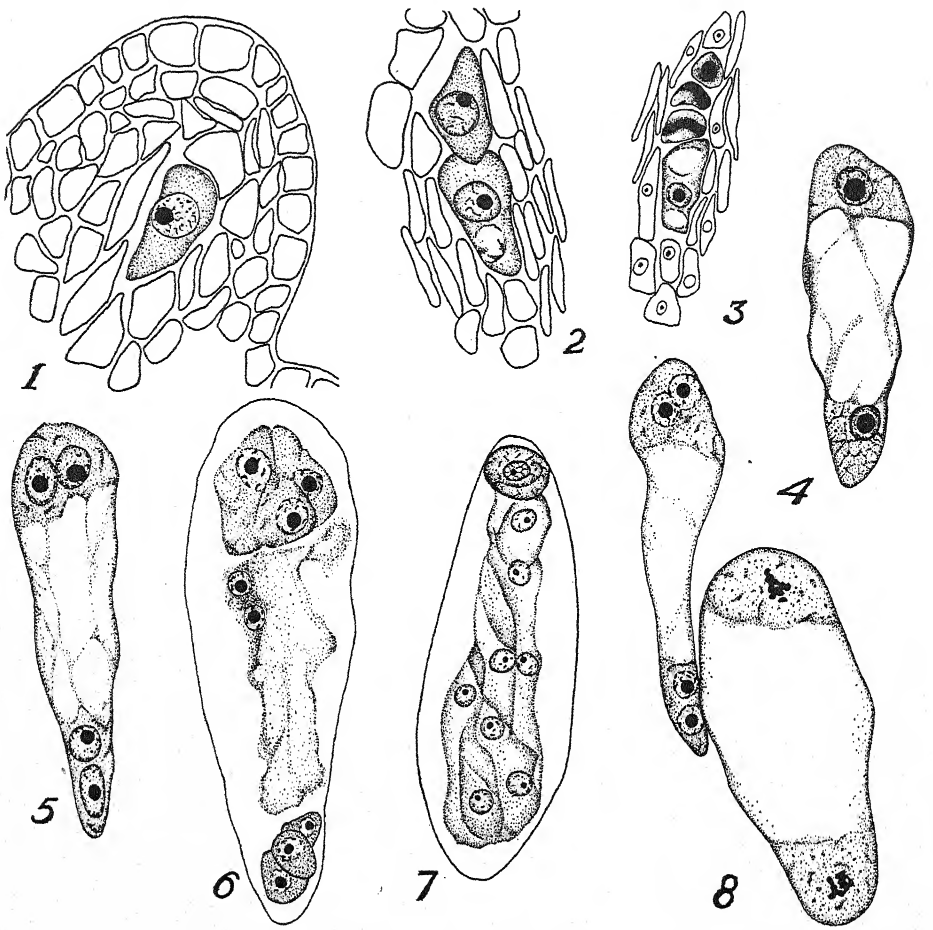
Fixation of the ovaries, which were dissected to insure a better infiltration of the fixing fluid, was made in Craf (7). After 24 hours the material was washed in 70 per cent alcohol, dehydrated by the butyl-alcohol method, and imbedded in paraffin. Sections were cut at 10–25 μ and stained in Heidenhain's haematoxylin.

Observations

MEGASPOROGENESIS.—The megaspore mother cell gives rise to a linear tetrad, and the three micropylar megaspores degenerate, the chalazal one forming the functional megaspore, from which a normal megagametophyte (figs. 1–6) develops. There are no differences between the two species studied. Two well-developed synergids occur near the micropyle. The egg lies between the two syner-

gids, a little farther away from the micropyle; in size it is similar to the synergids, except that its cytoplasm contains some inclusions absent in the former. The two polar nuclei are located in the middle of the megagametophyte; usually they remain separated until fertilization, sometimes fusing a little earlier. The three small antipodals degenerate very early.

As a general rule, the first meiotic division (fig. 1) occurs when the ovule is not yet fully developed. The megagametophyte is mature a few days before or by the day the flower opens (fig. 6). In some instances, however, opened flowers have the megagametophyte in the one-, two-, and four-nucleate stages. Usually megasporocytes and megagametophytes

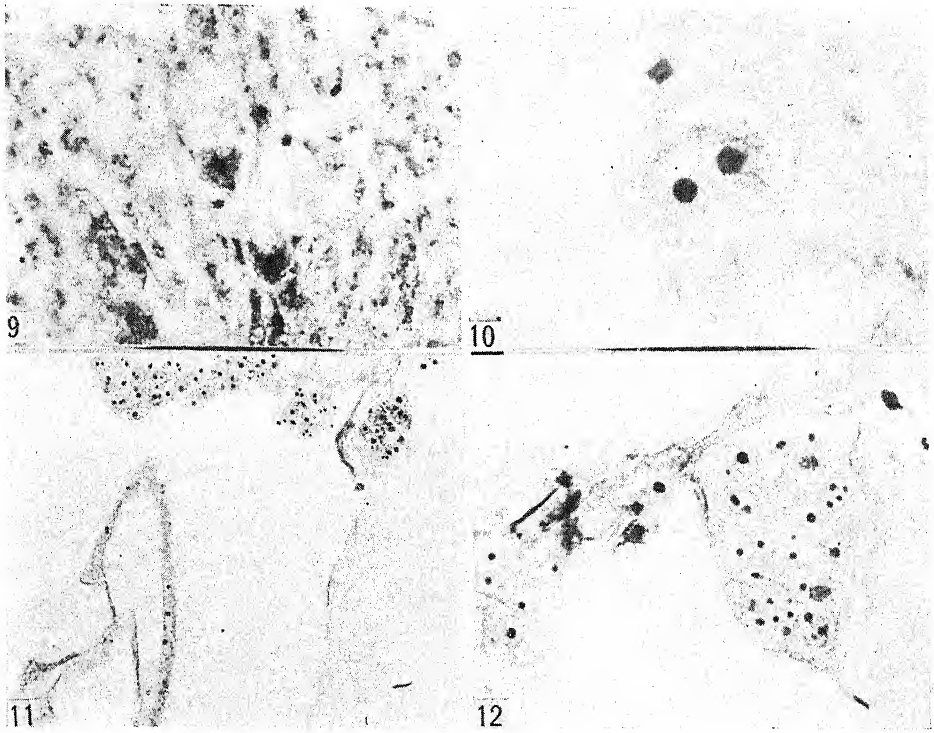


FIGS. 1–8.—Fig. 1, Grapefruit Foster: megasporocyte in prophase from ovary of small flower bud. Fig. 2, Grapefruit Foster: two megaspores in ovary of small flower bud. Fig. 3, Sour Orange: four megaspores, three micropylar ones degenerating; ovary of large flower bud. Fig. 4, Grapefruit Foster: megagametophyte with two nuclei; ovary of large flower bud. Fig. 5, Grapefruit Foster: megagametophyte with four nuclei; ovary of large flower bud. Fig. 6, Grapefruit Foster: complete megagametophyte; ovary of flower bud the day it opened. Fig. 7, Grapefruit Foster: egg cell in resting stage and endosperm with 8 nuclei; ovary 15 days after pollination. Fig. 8, camera-lucida drawing of fig. 9, including observations of adjoining sections. Figs. 1–6, 8, $\times 1000$; fig. 7, $\times 500$.

in different stages of development are found in the same ovary.

FERTILIZATION.—STRASBURGER (10) and OSAWA (6) state that fertilization usually occurs 4 weeks after pollination. In the Grapefruit Foster it was established that the period between pollination and fertilization averaged 4 days. This agrees with COIT's (3) statement that "the time required for complete fertilization after pollination varies with the varieties from thirty hours in the Satsuma Orange to four weeks in the trifoliate orange."

The pollen tube enters the megagametophyte through the micropyle, usually disorganizing one of the synergids. Sometimes both of them disappear at this time. In rare instances they remain untouched. The antipodals soon degenerate; the zygote then initiates a rather long resting period of about 50 days. This period has been found by OSAWA (6) to be 3–4 weeks in *Poncirus trifoliata.*



FIGS. 9–12.—Grapefruit Foster: Fig. 9, development of two megagametophytes in same ovule; ovary day the flower opened. Fig. 10, nucellar cell after first division giving rise to adventitious embryo; ovary 67 days after pollination. Fig. 11, nucellar embryos and endosperm nuclei; ovary 67 days after pollination. Fig. 12, detail of developing nucellar embryo; ovary 67 days after pollination. Figs. 9, 12, ×500; fig. 10, 1000; fig. 11, ×250.

Soon after its formation, through fusion of the polar nuclei with one of the generative nuclei from the pollen tube, the primary endosperm nucleus divides, and successive divisions result in a free nuclear endosperm (figs. 7, 11). Examination of ovaries collected up to 67 days after pollination showed that in some cases the endosperm nuclei numbered more than 1500. Later stages of endo-

sperm development were not examined, so that the formation of cell walls and subsequent cellular divisions reported by OSAWA (6) and by STRASBURGER (10) were not observed.

POLYEMBRYONY.—When the first divisions of the zygote occur to form the embryo resulting from fertilization, several nucellar cells (fig. 10) in the vicinity of the embryo sac, and usually close to the micropyle, through a series of divisions form a number of adventitious embryos (figs. 10–12), which penetrate the cavity of the embryo sac. Such a process, described by others in detail (10, 6), has been observed in young seeds of both varieties studied.

In an examination of many ovules, four instances were detected in which two normal gametophytes developed in the same ovule; the fertilization of their egg cells would therefore have given rise to the development of two generative embryos in the same seed. Figures 8 and 9 show stages of the development of such supernumerary gametophytes.

The occurrence of two gametophytes in the same ovule has already been described for several other genera: *Hiptage* (11), *Medicago* (8), *Poa* (1), and *Saxifraga* (2). With the exception of FROST'S (4, 5) identical twin hybrid *Citrus* seedlings, it is not known whether the other groups of hybrids derived from single seeds, as obtained by TRAUB and ROBINSON (14) and by SWINGLE, in 1909, were genetically identical.

To establish a hypothesis regarding the origin of twin hybrids, it is necessary first to determine whether they are genetically identical. If not, the occurrence of two gametophytes in the same ovule is the best explanation. When more than two hybrid seedlings develop from the same seed, their origin can be accounted for either through cleavage polyembryony alone or through this phenomenon combined with the existence of two gametophytes in the same ovule, as the occurrence of more than two is highly improbable.

Summary

1. A general review is presented of megasporogenesis, fertilization, endosperm formation and polyembryony in *Citrus*, most of the findings of STRASBURGER (10) and OSAWA (6) having been confirmed through investigation of *C. paradisi* Macf. and *C. aurantium* L.

2. A new form of polyembryony is presented, caused by the existence in some instances of two gametophytes in the same ovule. The origin of two nonidentical hybrids from the same seed is thus explained.

3. Three forms of polyembryony are therefore known in *Citrus*: (*a*) nucellar embryony, giving rise to a variable number of identical, "maternal" seedlings derived from the nucellus; (*b*) cleavage polyembryony (4, 5) originating through fission of the generative embryo; and (*c*) polyembryony caused by the occurrence of more than one normal gametophyte in the same ovule. The endosperm in *Citrus* is free.

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LITERATURE CITED

1. AKERBERG, ERIK, Apomictic and sexual seed formation in *Poa pratensis*. Heriditas **25**:359–370. 1939.
2. CHAPMAN, MARJORIE, The ovule and embryo sac of *Saxifraga virginiensis*. Amer. Jour. Bot. **20**:151–158. 1933.
3. COIT, J. E., Citriculture. Univ. Calif. Agr. Exp. Sta. Ann. Rep. 105–106. 1913–14.
4. FROST, H. B., Polyembryony, heterozygosis and chimeras in *Citrus*. Hilgardia **1**:365–402. 1926.
5. ———, The genetics and cytology of *Citrus*. Current Sci., special number on genetics. Pp. 24–27. 1938.
6. OSAWA, I., Cytological and experimental studies in *Citrus*. Jour. Coll. Agr. Imp. Univ. Tokyo **4**:83–116. 1912.
7. RANDOLF, L. F., A new fixing fluid and a revised schedule for the paraffin method in plant cytology. Stain Technol. **10**:85–86. 1935.
8. REEVES, R. C., Development of the ovule and embryo sac of alfalfa. Amer. Jour. Bot. **17**: 239–246. 1930.
9. SOKOL'SKAJA, B. P., On polyembryony in seeds of *Citrus*. Soviet Subtropics 44:66–67. 1938. (Pl. Breed. Abstr. **9**:444. 1939.)
10. STRASBURGER, E., Ueber Polyembryonie. Jenaische Zeitschr. Naturw. **12**:647–667. 1878.
11. SUBRA RAO, A.M., A note on the development of the female gametophyte of some Malpighiaceae and polyembryony in *Hiptage madablota*. Curr. Sci. (Bangalore) **6**:280–282. 1937.
12. TORRES, J. P., Polyembryony in *Citrus* and study of hybrid seedlings. Philip. Jour. Agr. **7**:37–58. 1936.
13. TRAUB, H. P., Artificial control of nucellar embryony in *Citrus*. Science **83**:165–166. 1936.
14. TRAUB, H. P., and ROBINSON, T. R., Improvement of subtropical fruit crops: *Citrus*. U.S. Dept. Agr. Yearbook 794. 1937.
15. WEBBER, H. J., Complications in *Citrus* hybridization caused by polyembryony. Science **11**:308. 1900.
16. ———, The influence of pollination on the set of fruit in *Citrus*. California Citrogr. **16**:304. 1930.
17. ———, The economic importance of apogamy in *Citrus* and *Mangifera*. Proc. Amer. Soc. Hort. Sci. **28**:57–61. 1931.
18. WEBBER, J. M., Polyembryony. Bot. Review **6**:575–598. 1940.

STUDIES ON AMBROSIA: III. PISTILLATE AMBROSIA ELATIOR × A. TRIFIDA AND ITS BEARING ON MATROCLINIC SEX INHERITANCE[1]

KENNETH L. JONES

Introduction

In nature *Ambrosia elatior* L. is predominantly monoecious. The staminate flowers are borne in small heads arranged in racemes, and the pistillate flowers occur in the axils of leaves or bracts. Rarely the racemes are pistillate or androgynous. The writer has shown (4) that the inheritance of floral types is controlled by the female parent. For example, the common monoecious type breeds true generation after generation, irrespective of the genotype of the pollen parent, and pistillate plants always yield a mixed progeny of monoecious, intergrade, and pistillate. On the other hand, *A. trifida* L. is strictly monoecious in nature, identical with the common type of *A. elatior*.

WYLIE (7) reported a natural hybrid between the species. On the basis of this compatibility, supported by success in crossing *A. bidentata* and *A. trifida* (3), interspecific crosses were attempted to study the inheritance of the pistillate character. It was planned to cross reciprocally *A. trifida* and nearly pistillate *A. elatior*, but attempts to use *A. elatior* as a male parent have failed for 3 consecutive years. The cross reported here between pistillate *A. elatior* and *A. trifida* gives additional evidence of the peculiar matroclinic sex inheritance in *A. elatior* and incidentally offers interesting material for meiotic studies because of specific differences in the size and number of chromosomes.

Results

Seventy pistillate plants of *A. elatior* were grown amidst monoecious *A. trifida* in the greenhouse at the University of Michigan. Planting was sufficiently early to avoid the "ragweed season" in nature. Only seven seeds developed, and these were after-ripened at 5° C., as DAVIS (1) found necessary for this genus. The seeds were sown in early May, together with some of the parent species.

The foliage of the hybrid plants was clearly intermediate in type. Figures 1–3 show typical leaves of the parent species and of a hybrid, from plants grown under similar conditions in the greenhouse. The three-lobed habit of *A. trifida* is evident in the hybrid, but the lobes are considerably reduced and the terminal one pinnatifid. Some leaves have the serrate margin of *A. trifida*. The surfaces are puberulent, the lower surface slightly lighter in color.

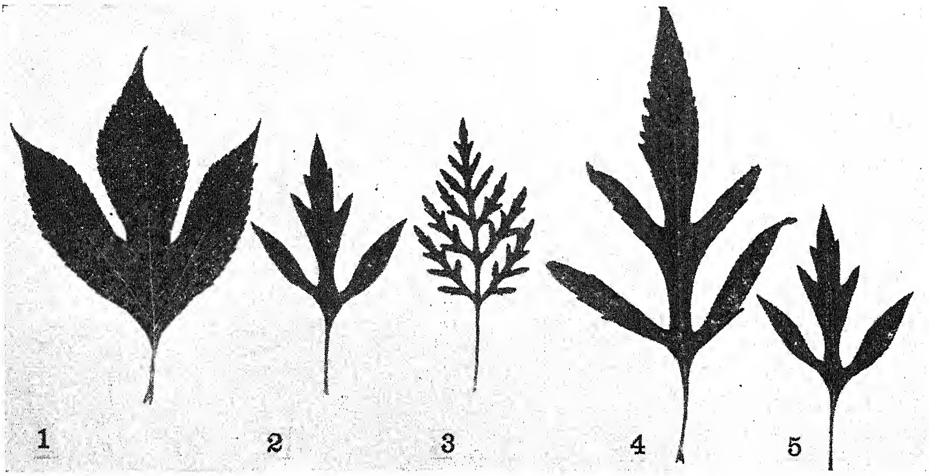
WYLIE (7) illustrated leaves of a natural hybrid collected near Iowa City. They were 2 dm. long and 1.5 dm. wide and were 3-divided with pinnatifid segments. The writer also found a specimen of a natural hybrid on the bank of the Huron River near Ann Arbor in 1936. One of its leaves (fig. 4) is shown with a leaf of an artificial hybrid (fig. 5) for comparison. Its leaves were about 1.5 dm. long and 0.8 dm. wide, whereas the artificial hybrids were only 1 × 0.6 dm.

Chlorotic areas appeared in leaves of all plants from the outset. The deficiency was slight in plants 1, 5, and 7; more marked in 2, 4, and 6; and extreme in plant 3. Plants 1 and 6 had wrinkled leaves which suggested a mosaic disease. These abnormalities were perhaps the effects of recessives for which there were no allelomorphs.

The hybrids grew slowly. On July 11,

[1] Paper from the Department of Botany, University of Michigan, no. 731.

A. trifida was 6.5–7 dm. tall and *A. elatior* mostly about 3.0 dm., whereas the hybrids were only 1.4–3.0 dm. Pollination began on July 1 for *A. elatior*, July 9 for *A. trifida*, and August 17 for the hybrids. On July 21, hybrid plants 1, 2, 3, 4, and 7 were transplanted to the field with those of the parent species. The maximum heights at the end of the season were: *A. trifida* 9 dm. in the field and 12 dm. in the greenhouse; *A. elatior* 4.4 and 7 dm.; and the hybrids 5.5 dm. under both environments. The poor growth of the parent species outdoors was due to their maturity when transplanted. All hybrid plants were self sterile and failed to produce seed when pollinated by the parent species. The natural crosses observed by WYLIE and the writer also formed no seed.



FIGS. 1–5.—Fig. 1, *A. trifida*. Fig. 2, *A. elatior*×*A. trifida*. Fig. 3, *A. elatior*. Fig. 4, natural hybrid. Fig. 5, *A. elatior*×*A. trifida*.

The plants were of three sex types, exactly comparable with the offspring of a pistillate plant within the species *A. elatior*, namely: (*a*) pistillate (fig. 7); (*b*) monoecious with staminate racemes (fig. 8); and (*c*) monoecious intergrades with androgynous racemes (figs. 10–14). For convenience, the types will be designated pistillate, monoecious, and intergrade. The great diversity of the intergrades is illustrated in figures 10–14, in which their racemes are arranged in a progressively staminate series, as follows: figure 10, pistillate except for a few small staminate heads at apex; figure 11, pistillate except in upper left portion; figure 12, pistillate and staminate in about equal proportions—the pistillate in lower half; figure 13, staminate except for a cluster of pistillate heads in submedian portion; and figure 14, staminate but for two small pistillate heads at apex.

HYBRID PLANTS

PLANT 1, PISTILLATE.—Heads subtended by leafy bracts 2 cm. long (fig. 7); in some racemes the terminal 0.5 cm. lacked bracts. Racemes 5–7 cm. long. Height 4.4 dm.

PLANT 2, INTERGRADE.—Racemes predominantly staminate. Most racemes staminate except for a few sterile pistillate heads at apex (fig. 14). Some entirely staminate and others only half stami-

nate, with pistillate heads subtended by long bracts (fig. 12). Racemes 6 cm. long. Height 5.5 dm.

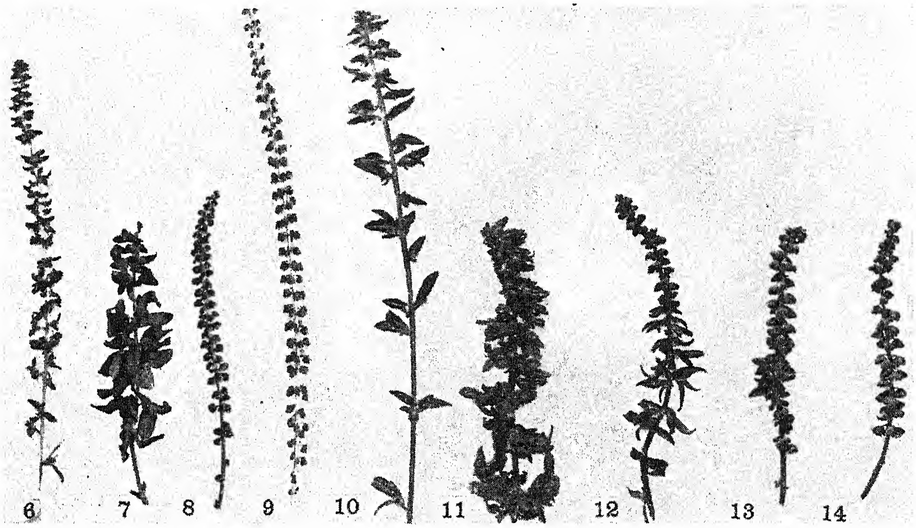
PLANT 3, PISTILLATE.—Poorly developed plant with general habit like plant 1. Leaves extremely chlorotic. Height 4.4 dm.

PLANT 4, INTERGRADE.—Racemes predominantly staminate. A few inflorescences had occasional pistillate heads; these were never terminal and seldom bore long bracts. This plant most closely resembled *A. elatior*. Racemes 8 cm. Height 3.5 dm.

PLANT 5, INTERGRADE.—Racemes predominantly pistillate. The only staminate heads occurred at the apex of one short raceme. Pistillate heads all subtended by short bracts, less than 1 cm. in length. Racemes 18 cm. Height 4.8 dm.

PLANT 6, INTERGRADE.—Racemes predominantly pistillate. The only staminate heads occurred in lower portion of central raceme. Pistillate heads seldom subtended by bracts. Height 5.5 dm.

PLANT 7, MONOECIOUS.—Racemes entirely staminate (fig. 8), pistillate flowers in leaf axils. Racemes 11 cm. long. Height 4.6 dm.



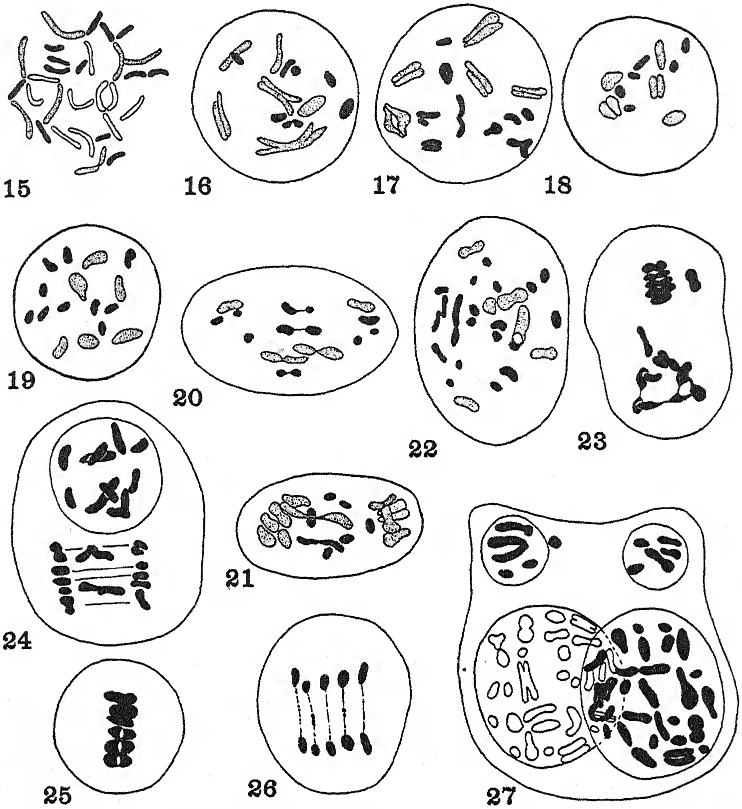
FIGS. 6–14.—Figs. 6–9, racemes of *A. elatior*×*A. trifida* and parents. Fig. 6, pistillate raceme of *A. elatior*. Fig. 7, same of hybrid (plant 1). Fig. 8, staminate raceme of hybrid (plant 7). Fig. 9, same of *A. trifida*. Figs. 10–14, androgynous racemes of hybrids in progressively staminate series. Fig. 10, pistillate except for a few staminate heads at apex. Fig. 11, pistillate except in upper left portion. Fig. 12, pistillate in lower half and staminate in upper. Fig. 13, staminate except for submedian portion. Fig. 14, staminate except for two heads at apex.

CYTOLOGY

Root tips were fixed in Navashin's and Bouin's reagents and the sections treated with iron-alum haematoxylin. Satisfactory cytological material was obtained, except for plant 6. The writer previously reported (4, 3) that *A. elatior* has a haploid chromosome number of 18 and *A. trifida* 12, with the chromosomes larger in the latter species. The somatic figures confirmed the hybrid origin. For example, figure 15 is a typical late prophase with 30 chromosomes, of which 13, filled in solid, are identifiable as *A. elatior*, and 7, stippled, as *A. trifida*. Somatic counts were as follows: plant 1, 26–31

chromosomes; plant 2, 29; plant 3, 30–33; plant 4, 26–31; plant 5, 28–30; and plant 7, 31. Exact counts were often impossible because of the overlapping of curved segments.

Racemes of plants 2 and 7 were fixed in Carnoy's fluid and sections stained with crystal violet-iodine. No attempt was made to interpret early prophases. Diakinesis revealed regularly an autosynapsis within each species. For *A. trifida* this was very marked; often only bivalents were found. The writer is uncertain that autosynapsis is ever complete



FIGS. 15–27.—Fig. 15, prophase of somatic mitosis in root tip. Thirty chromosomes: 13 filled in solid (*A. elatior*); 7 stippled (*A. trifida*); and 10 clear (unidentifiable). Figs. 16–22, meiotic division I, showing portion of chromosomes only. *A. trifida* stippled and *A. elatior* solid. Fig. 16, diakinesis; 4 bivalents of *A. trifida*, 1 with heteromorphic chromosomes. Possible instance of allosynapsis. Fig. 17, diakinesis; 5 bivalents of *A. trifida* and also of *A. elatior;* 4 univalents of *A. elatior*. Fig. 18, typical polar view of metaphase. Fig. 19, metaphase in polar view showing 6 chromosomes of *A. trifida* and 10 of *A. elatior*. Fig. 20, anaphase: 5 autosynaptic mates undergoing separation, 2 of *A. trifida* and 3 of *A. elatior*. Near each pole are 4 univalents, 1 of *A. trifida* and 3 of *A. elatior* which presumably have disjoined. Fig. 21, anaphase; 5 univalents of *A. trifida* at each pole, and 1 bivalent undergoing separation. Eight chromosomes of *A. elatior;* 1 bivalent being separated and 6 univalents disjoined earlier. Fig. 22, anaphase; disjunction of chromosome of *A. elatior* from end of its *A. trifida* partner. High chromosome number (21) of *A. elatior* indicates division of unpaired univalents. Figs. 23–27, meiotic division II; all chromosomes filled in solid since size differences were slight except in telophase. Fig. 23, metaphase above, prophase below. Fig. 24, lagging of chromosomes. Fig. 25, metaphase, illustrating regularity of division II. Fig. 26, anaphase; almost diagrammatically equational. Fig. 27, telophase; total of 70 chromosomes indicates division of some univalents in I and II; irregularity of one division and equational nature of other corroborated.

in *A. trifida*, since the sections were too thin to show entire nuclei and the highest number of bivalents observed was 5 (fig. 17). Also, allosynapsis occurred infrequently. Pairing within *A. elatior* was less common, but there were always some bivalents, up to as many as 5 (fig. 17). Synaptic mates were usually similar but heteromorphism was not rare (figs. 16, 17).

The first meiotic division was very irregular and extremely difficult to interpret. The division involved primarily a separation of autosynaptic mates and a random segregation of unpaired univalents. A few univalents underwent equational division in certain microsporocytes. Characteristic polar views of metaphase revealed only a portion of the total number of chromosomes (fig. 18). Figure 19 is unusual in this respect, since 16 chromosomes appear, including 6 of *A. trifida* and 10 of *A. elatior*. The most critical observation made for division I is illustrated in figure 20. At the equator are 5 autosynaptic bivalents undergoing separation, 2 of *A. trifida* and 3 of *A. elatior;* near each pole are 4 univalents, 1 of *A. trifida* and 3 of *A. elatior*, which presumably have disjoined. Figure 21 is less convincing. At each pole are 5 univalents of *A. trifida*, 1 bivalent of *A. trifida* is under the stress of disjunction, and the rest of the field shows 8 chromosomes of *A. elatior;* 1 bivalent is being separated and 6 univalents have apparently disjoined earlier. Figure 22 illustrates one of the few instances of the separation of allosynaptic mates. A small chromosome of *A. elatior* is disjoining from the end of its *A. trifida* partner. Lagging of chromosomes occurred rarely, except in certain anthers where it was extreme in every meiocyte. Amitosis was similarly restricted, so that localized physiological phenomena must bring on these abnormalities.

Meiotic division II seemed to follow directly without delicate reticula developing in the interphase. Figure 23 shows one of the few prophases with anastomoses. The second division is as regular as the first is irregular. The chromosomes line up with diagrammatic neatness at the equatorial plate, and all undergo equational division at almost the same moment (figs. 25, 26). The chromosomes are much shortened and thickened, so that they cannot be identified as to species. The only aberrant phenomenon was an occasional lagging of chromosomes (fig. 24).

The telophase nuclei were grouped usually in quartets, with sister nuclei having approximately the same chromosome numbers. In about 50 per cent of the quartets, one pair of nuclei had very different chromosome numbers from the other. The total number of chromosomes within a quartet seldom exceeded 60, which confirmed the observation that unpaired univalents rarely divide in division I. Figure 27 shows an exceptional instance in which 70 chromosomes occurred; it also illustrates the irregularity of one division and the equational character of the other. Four pollen grains of unlike size ordinarily developed from a sporocyte.

Discussion

The writer has reported (4) that the sex inheritance in *A. elatior* is controlled by the female parent. For example, a pistillate plant, regardless of the genotype of the male, produces a high proportion of pistillate offspring and a motley assemblage of monoecious segregates. It is significant that these results were obtained when pistillate plants were crossed with *A. trifida*, which in nature is strictly of one monoecious type.

MANN (6) has reported that a short photoperiod may cause pistillate flowers

of *A. trifida* to develop in positions usually occupied by staminate, and he states that "In *A. trifida*, on the other hand, length of photoperiod and number of photoinductive cycles have a pronounced effect on sex expression. Lacking experimental information, the uniformity in field plants would suggest that genetic factors are of less importance in *A. trifida* than in *A. elatior*."

Broad comparisons of the relative importance of environment and heredity are always fraught with difficulty. The uniformity of *A. trifida* in the field may be as genetical in its causes as is the variability of *A. elatior*. From the environmental angle, the experimental work has not been comparable, but there is evidence that *A. elatior* of certain strains is alterable in sex. GARNER and ALLARD (2) grew plants under a photoperiod of 7 hours from June 3 to July 1, when pollination began in normal staminate racemes. The plants were restored to natural light conditions and blossomed a second time in August, producing "pistillate flowers almost exclusively." The writer (5) grew known genetical strains under long photoperiods and found that some were altered and others were not. Strain P, for example, showed an increase of 24 per cent pistillate plants under a photoperiod of 20 hours. This was perhaps equal to the most extreme case cited by MANN for *A. trifida*, "in which some or all staminate involucres were replaced by pistillate involucres" in thirteen terminal racemes and eight lateral out of 100 plants.

Deviations were always toward the pistillate condition, and certain unusual modifications occurred in both species, such as the development of leaves in the staminate racemes (cf. 6, fig. 4*B* and 5, fig. 16). The number of hybrid plants was too small for genetical analysis; however, the wide range of types suggests that sex is of a quantitative nature, influenced by many genes. There is no question of the transmission of the pistillate character by the female gametes of *A. elatior*.

The following chromosome numbers have been determined by the writer for *Ambrosia: A. trifida* n = 12, *A. bidentata* n = 17, *A. elatior* n = 18, *A. trifida* × *A. bidentata* 2n = 29 (3), and *A. trifida* × *A. elatior* 2n = 30. On the basis of these numbers and of the rather complete autosynapsis among chromosomes in the haploid complement of *A. trifida*, it is probable that the basic number for the genus is 6.

Summary

1. Pistillate plants of *A. elatior* always yield a mixed progeny of monoecious plants, intergrades, and pistillate individuals, irrespective of the genotype of the pollen parent.

2. Pistillate plants were crossed with *A. trifida*, which is strictly monoecious in nature. Seven hybrids were secured: two pistillate, four intergrades, and one normal monoecious.

3. The hybrids had approximately 30 somatic chromosomes (*A. trifida* n = 12, *A. elatior* n = 18). The first meiotic division was extremely irregular and involved primarily a separation of autosynaptic mates and a random segregation of univalents. The second division was regular and equational.

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LITERATURE CITED

1. DAVIS, W. E., Primary dormancy, after-ripening, and development of secondary dormancy in embryos of *Ambrosia trifida*. Amer. Jour. Bot. **17**:58–77. 1930.
2. GARNER, W. W., and ALLARD, H. A., Effect of the relative length of day and night and other factors of the environment on growth and reproduction in plants. Jour. Agr. Res. **18**:553–606. 1920.
3. JONES, K. L., *Ambrosia bidentata* Michx.×*A. trifida* L. Amer. Mid. Nat. **14**:720–724. 1933.
4. ———, Studies on *Ambrosia*. I: The inheritance of floral types in the ragweed, *Ambrosia elatior* L. Amer. Mid. Nat. **17**:673–699. 1936.
5. ———, Studies on *Ambrosia*. II: Effect of certain environmental factors on floral development of *Ambrosia elatior*. BOT. GAZ. **98**:296–305. 1936.
6. MANN, L. K., Effects of photoperiod on sex expression in *Ambrosia trifida*. BOT. GAZ. **103**:780–787. 1942.
7. WYLIE, R. B. A hybrid ragweed. Proc. Iowa Acad. Sci. **22**:127–128. 1915.

EFFECTS OF TEMPERATURE ON RUBBER ACCUMULATION BY THE GUAYULE PLANT

JAMES BONNER

Introduction

Preliminary experiments in which plants of guayule (*Parthenium argentatum*) were cultivated in the greenhouse under various conditions for varying periods showed that such plants failed to form any considerable amount of rubber; generally it was less than 0.5% of the dry weight of the defoliated plant. The following environmental factors failed materially to increase the percentage of rubber in such greenhouse plants: (1) drought of varying degrees, (2) deficiency of nitrogen to varying extents, (3) day length. The data to be presented here, however, indicate that the temperature to which the plants are subjected is an important factor in influencing rubber formation by young plants.

MATERIAL AND METHODS.—All plants for this investigation were grown from seedlings supplied by the Intercontinental Rubber Company or from nursery stock[1] supplied by the U.S. Forest Service. The plants were planted in 4-mesh gravel, contained in 2-gallon glazed crocks, or in 1- or 2-gallon cans previously painted with a nontoxic asphaltum paint. All plants were supplied daily with Hoagland's nutrient solution (complete) and watered as needed with tap water. Plants were in part greenhouse-grown and in part grown outdoors (during the summer months).

Controlled temperature conditions were achieved in air-conditioned greenhouses, where the temperature was regulated to $\pm 1°$ F., and in a glass-roofed insulated box cooled with a 2-ton compressor through direct expansion coils and in which the temperature could be regulated to $\pm 1°$.

Rubber determinations were made on material dried rapidly at 65° C. and ground in a Wiley mill. Two-gm. aliquots of the material were then extracted with absolute acetone for 16 hours. The acetone-soluble material is referred to as "resin." The acetone-insoluble residue was then extracted with benzene for 8 hours, the benzene removed, concentrated, and the solid residue dried and weighed. The benzene-soluble portion is referred to as "rubber." The rubber so obtained is impure, particularly when its concentration in the plant is under 0.5%. The determination is highly reproducible, however, and the results are of value from a comparative standpoint, even at low productive levels.

All plants were defoliated at the time of harvest and the leaves weighed separately from the stems and roots. Since the leaves contain but little rubber, the analyses given are based on the stem and root samples.

Experimentation

On January 2, 1942, a group of plants (grown from Intercontinental Rubber Company seedlings) $2\frac{1}{2}$ months old from the time of transplanting from the seedling flat was selected for uniformity and randomized into four lots of twelve plants each. Of these groups, one was harvested as an initial control, one was placed in a controlled-temperature greenhouse at 80° F.[2] (day and night), one remained in the ordinary greenhouse, and one was removed to an open unshaded position outdoors. The average daily

[1] Owing to the critical shortage of guayule plants in the spring of 1942, the U.S. Forest Service was able to supply only cull nursery stock.

[2] All succeeding temperature references are Fahrenheit.

temperature in the greenhouse during the 2 months of the experiment was 72°, while the nightly minimum was 56°. Outdoors during the same period the average daily maximum temperature was 63°, while the average nightly minimum was 43°. At the expiration of 2 months all plants were harvested, composited in groups of two plants, dried, ground, and assayed for resin and rubber. The results of this experiment (G-25) are given in table 1. The plants increased markedly in dry weight during the 2 months under all conditions, although growth was greater in the greenhouses than outdoors. In the two greenhouses the percentage of rubber decreased significantly during the 2 months, although the total amount per plant increased. In the plants which remained outdoors, however, the percentage of rubber almost trebled over its initial value, rising to 1.5%, while the total amount per plant was double that of the larger plants which had remained in the greenhouse. These results in all probability were not due to the greater light intensity received by the outdoors plants, as can be seen from the experiment of table 2.

TABLE 1

GROWTH AND RUBBER FORMATION OF GREENHOUSE-GROWN GUAYULE PLANTS, JANUARY AND FEBRUARY, 1942. EXPERIMENT G-25. ANY COMPARISON ATTENDED BY 10° OF FREEDOM

Material	Dry wt. per 2 plants, stems and roots (gm.)	Resin (%)	Rubber (%)	Rubber per 2 plants (mg.)
Initial plants	1.25±0.081	2.82±0.089	0.57±0.050	7
2 months at 80° F. (day and night)	8.33± .48	3.06± .090	0.36± .046*	30
2 months in ordinary greenhouse (ave. day max. 72°, night min. 55°)	7.25± .28	3.04± .162	0.40± .042*	29
2 months outdoors (ave. day max. 63°, night min. 43°)	3.83±0.28	4.07±0.043	1.52±0.066†	58

*Differs significantly from that of initial plants.
† Differs highly significantly from all others.

For this experiment (G-54), plants grown from cull nursery seedlings (spring, 1942, stock of the Intercontinental Rubber Company supplied by U.S. Forest Service) were used. These were grown outdoors in gravel contained in 1-gallon cans. On September 28, 100 plants selected for uniformity were randomized in five lots of twenty plants each. One lot was maintained in a greenhouse for 2 months and a second lot for 4 months. A third lot was maintained outdoors for 2 months in a lath-house, where the light intensity approximated that in the greenhouse. The fourth lot was maintained outdoors for 4 months, and the remaining lot served as the initial harvest. At the time of harvest each lot of twenty plants was harvested as ten composite groups of two plants each. During the first month of the experiment the heating system of the greenhouse failed to function, and the average nightly minimum temperature was approximately 50°. During the last 3 months of the experiment the average minimum nightly greenhouse temperature was 60°. As shown in table 2, the plants maintained outdoors steadily increased in rubber

concentration during the 4 months of the experiment; those in the greenhouse increased in percentage during the first 2 months but decreased during the second period. At the end of the experiment the outside plants had 4.5 times as high a rubber concentration as the plants maintained in the greenhouse.

A further experiment (G-26) also makes it appear unlikely that the results of tables 1 and 2 were due to lower light intensity in the greenhouse. For this experiment, use was made of plants which had been planted in the field in June, 1941. In a small portion of the field, thirty-six plants selected for uniformity were randomized in three lots of twelve plants each. Twelve plants were harvested as an initial control, twelve more were transplanted to 12-inch pots and removed to a greenhouse, while twelve were allowed to remain in the field. The twelve plants moved to the greenhouse were placed near a door which was kept open, so that the temperature approximated that outside. After 2 months the plants were analyzed (table 3). Although the transplanted plants made less growth than those which remained in the field, their increase in percentage of rubber was comparable with that of the field plants.

TABLE 2

GROWTH AND RUBBER FORMATION OF YOUNG GUAYULE PLANTS, SEPTEMBER 28, 1942—FEBRUARY 1, 1943. EXPERIMENT G-54. ANY COMPARISON ATTENDED BY 18° OF FREEDOM

Material	Dry wt. per 2 plants (gm.)		Resin (%)	Rubber (%)
	Stems and roots	Leaves		
Initial plants	3.66±0.24	5.22±0.22	4.35±0.154	0.53±0.034
In greenhouse 2 months	7.78± .43	8.39± .56	4.24± .114	1.03± .087
In greenhouse 4 months	11.23± .58	7.72± .27	3.48± .123	0.85± .062
Outdoors 2 months	8.78± .56	7.42± .52	4.69± .075	2.69± .105
Outdoors 4 months	10.14±0.44	4.77±0.29	4.92±0.102	3.79±0.159

The experiments of tables 1, 2, and 3, taken together, suggest that temperature may influence increase in rubber concentration in guayule. The following experiments support such a supposition.

VARYING NIGHT TEMPERATURES.—For experiment G-39, a group of 100 greenhouse-grown plants (from seedlings supplied by the Intercontinental Rubber Company) selected for uniformity were divided into five lots. One lot was harvested immediately as initial control. Two lots were placed in a greenhouse maintained at 80°. Two lots were transferred to a similar greenhouse where the day temperature was maintained at 80° (8 hours) and the night temperature at 60° (16 hours). After 2 months one lot of twenty plants was harvested from

TABLE 3

GROWTH AND RUBBER FORMATION OF FIELD-GROWN GUAYULE PLANTS, JANUARY AND FEBRUARY, 1942. EXPERIMENT G-26. ANY COMPARISON ATTENDED BY 10° OF FREEDOM

Material	Dry wt. per 2 plants, stems and roots (gm.)	Resin (%)	Rubber (%)
Initial plants	84.7±2.75	4.24±0.050	1.63±0.120
In field 2 months	122.4±4.13	5.47± .121	4.50± .148
In greenhouse at outside temperature 2 months	94.8±2.77	5.19±0.219	4.38±0.160

each temperature condition. There was no significant change in percentage rubber in either lot from the concentration found in the initial plants (table 4). The night temperature in the one greenhouse room was then changed to 55° and the experiment continued for another month, after which the remaining two lots were harvested. The rubber percentage in the plants maintained at 80° day and night was now significantly lower than that of the initial plants. In no treatment of this experiment was there a significant increase over that in the initial plants.

In a second experiment (G-62) in night temperatures of 80° and 55°, no significant change occurred in rubber percentage in plants left for 1 month at a constant temperature of 80°. Plants subjected to a night temperature of 55° yielded a small increase (table 4).

For all but one of the next six experiments the refrigerated box was used. For experiment G-42, sixty plants selected for uniformity (grown from cull nursery stock supplied by the U.S. Forest Service) were divided into three lots of twenty each. One lot was harvested as an initial sample. Another lot was removed to the greenhouse at a constant temperature of 80°, and the third lot was shifted daily so that it had an 8-hour day in the greenhouse at 80° and a 16-hour night in the refrigerated box at 50°. After 1 month, these two lots were harvested and analyzed. Table 5 shows that the rubber concentration decreased significantly in the plants maintained at 80° day and night but increased significantly in the plants which received a 50° night temperature. In a second experiment (G-43) at a 50° night temperature, somewhat similar results were obtained. Plants which received continuous temperature of 80° did not increase in rubber concentration, although they did not decrease as in the previous experiment.

TABLE 4

GROWTH AND RUBBER FORMATION OF GREENHOUSE-GROWN GUAYULE PLANTS UNDER CONTROLLED TEMPERATURES. EXPERIMENTS G-39 AND G-62. ANY COMPARISON ATTENDED BY 18° OF FREEDOM

Exp. no.	Material	Dry wt. per 2 plants, stems and roots (gm.)	Resin (%)	Rubber (%)	Rubber per 2 plants (mg.)
G-39a	1. Initial plants	7.04±0.17	3.11±0.075	0.60±0.059	42
	2. 2 months (80° day and night)	24.2 ±0.67	3.22± .172	0.45± .033	109
	3. 2 months (80° day, 60° night)	27.6 ±1.28	3.40± .074	0.47± .021	130
G-39b	4. Plants of 2 (39a) additional month at 80°	30.8 ±1.63	3.42± .092	0.37± .030*	114
	5. Plants of 3 (39a) additional month at 80° day, 55° night	37.4 ±1.45	3.72± .009	0.51± .032	191
G-62	1. Initial plants	2.22±0.140	3.41± .182	0.37± .022	8.2
	2. 1 month (80° day and night)	3.22±0.135	3.22± .049	0.38± .029	12.2
	3. 1 month (80° day, 55° night)	3.34±0.165	3.34±0.063	0.48±0.033†	16.0

* Significantly lower than that of initial plants.
† Significantly higher than that of initial plants.

Plants given nights at 50° doubled in rubber percentage during the month of the experiment. Somewhat less growth with the cool nights occurred in this experiment than in the preceding, possibly because during the first night the cold box thermostat failed, and the temperature dropped to near freezing. In experiment G-43 (table 5), one lot of twenty plants was maintained at 50° continuously at 80° did not increase in rubber concentration, whereas plants which received a night temperature of 45° trebled in percentage and increased in total rubber by 5.7 times during the month of the experiment. Plants maintained continuously at 45° did not grow and apparently decreased in rubber concentration. Although the plants which received 45° night temperature increased

TABLE 5

GROWTH AND RUBBER FORMATION OF GREENHOUSE-GROWN GUAYULE PLANTS UNDER CONTROLLED TEMPERATURES. EXPERIMENTS G-42 AND G-43. ANY COMPARISON ATTENDED BY 18° OF FREEDOM

Exp. no.	Material	Dry. wt. per 2 plants, stems and roots (gm.)	Resin (%)	Rubber (%)	Rubber per 2 plants (mg.)
G-42	Initial plants	8.58±0.38	4.06±0.176	0.43±0.036	37
	1 month (80° day and night)	14.4 ± .74	3.56± .173	0.23± .020*	33
	1 month (80° day, 50° night)	14.7 ± .48	3.83± .135	0.62± .050†	91
G-43	Initial plants	4.45± .25	3.95± .082	0.29± .012	13
	1 month (80° day and night)	9.91± .67	4.12± .028	0.28± .019	28
	1 month (80° day, 50° night)	6.44± .20	4.00± .149	0.61± .097†	39
	1 month (50° day and night)	4.35±0.22	4.10±0.254	0.35±0.017	15

* Significantly lower than that of initial plants.

† Highly significantly greater than initial plants of this experiment.

day and night. This lot made no growth and formed no rubber. The lack of growth may have been due in part to the difficulty of achieving a high light intensity within the box and at the same time regulating the temperature to 50° during the day. In part, however, it appears to be correlated with adaptation of the plant to the low day temperature. Plants removed from a warm greenhouse and placed in the 50° box during the day wilted severely for several days, even when adequately supplied with water.

The experiments of table 5 were continued with two experiments in which plants were switched daily between a day temperature of 80° and a night temperature of 45°. As can be seen from table 6 (experiment G-50), plants maintained in dry weight of stems and of roots as much as did the plants which received a night temperature of 80°, still the growth habits of the two lots were entirely different. Those plants which received a high night temperature showed rapid shoot growth and made abundant growth of new leaves during the month of the experiment; those which received a low night temperature made little new shoot growth, produced few new leaves, and had a dormant appearance in contrast to the first group.

In experiment G-52, use was made of the same temperatures as in G-50. The starting material for both experiments consisted of plants grown outdoors during the late summer. They were removed to the greenhouse on September

28 and on this date contained 1.00% rubber, in contrast to the 0.50% contained by the similar plants of G-50 when they were removed to the greenhouse on September 18. In experiment G-52, the rubber concentration of the plants maintained at 80° at night decreased markedly during the month of the experiment; those at 45° increased rapidly in percentage rubber during the same time.

were in all cases similar to those given.) In table 8, the change in percentage of rubber during the experiment for plants maintained at 80° is variable but small. In four cases the percentage decreased significantly and in six cases it did not; in no case was any significant increase noted. It is to be stressed that—even in the cases of apparent decrease in rubber percentage—the absolute amount of rubber present per plant increased (table 8).

TABLE 6

GROWTH AND RUBBER FORMATION OF YOUNG GUAYULE PLANTS UNDER CONTROLLED TEMPERATURES. EXPERIMENTS G-50 AND G-52. ANY COMPARISON ATTENDED BY 18° OF FREEDOM

Exp. no.	Material	Dry wt. per 2 plants (gm.)		Resin (%)	Rubber (%)	Rubber per 2 plants (mg.)
		Stems and roots	Leaves			
G-50	Initial plants.........	5.43±0.33	6.46±0.42	4.33±0.082	0.50±0.030	27
	1 month (80° day and night)............	11.6 ± .59	17.5 ±1.07	3.62± .172	0.43± .018*	50
	1 month (80° day, 45° night)............	10.3 ± .34	11.3 ± .30	4.09± .099	1.49± .121†	153
	1 month (45° day and night)............	5.03± .16	5.81± .31	3.38± .116	0.40± .021‡	20
G-52	Initial plants.........	5.62± .432	6.82± .430	4.39± .091	1.00± .112	56
	1 month (80° day and night)............	11.40± .888	14.53±1.30	3.56± .092	0.71± .039‡	81
	1 month (80° day, 45° night)............	10.94±1.02	11.26±1.40	3.72±0.092	2.01±0.142†	220

* Not significantly different from that of initial plants.
† Highly significantly greater than any other in this experiment.
‡ Significantly smaller than that of initial plants.

The two experiments of table 7 were similar to those of table 6, except that the night temperature was maintained at 40°. Results qualitatively similar to those with 45° night temperature were obtained, although the response in rubber formation was less striking.

Table 8 presents a summary of the 1–2 months' experiments with controlled day and night temperatures. (In addition to the ten experiments tabulated, eight further tests not here reported were carried out at less well-controlled low temperatures. Three other experiments will be summarized separately. The results

In the three experiments with night temperatures of 60° and 55°, small or insignificant increases in concentration took place within the 1–2 months' period. At night temperatures of 50°, greater increases in rubber percentage were noted, and still greater were obtained when night temperatures were 45°. In the two experiments with night temperatures of 40°, the apparent response in rubber formation was smaller than with night temperatures of 45°. It may be that the optimum night temperature for rubber formation under the conditions used is approximately 45°.

TABLE 7

GROWTH AND RUBBER FORMATION OF YOUNG GUAYULE PLANTS UNDER CONTROLLED TEMPERATURES. EXPERIMENTS G-57 AND G-61. ANY COMPARISON ATTENDED BY 18° OF FREEDOM

Exp. no.	Material	Dry wt. per 2 plants (gm.)		Resin (%)	Rubber (%)	Rubber per 2 plants (mg.)
		Stems and roots	Leaves			
G-57	Initial plants.........	7.57±0.302	9.58±0.314	4.07±0.159	0.99±0.073	75
	1 month (80° day and night).............	11.37± .460	13.41± .610	3.32± .061	0.77± .064*	88
	1 month (80° day, 40° night).............	12.24± .519	13.46± .632	3.78± .136	1.65± .118†	202
	1 month (40° day and night).............	8.78± .331	10.52± .452	3.54± .050	0.96± .069	84
G-61	Initial plants.........	2.93± .145	2.87± .175	4.11± .148	0.40± .034	12
	1 month (80° day and night).............	4.80± .324	4.86± .310	3.54± .047	0.51± .038	24.5
	1 month (80° day, 40° night).............	4.83±0.176	4.25±0.161	3.49±0.101	0.85±0.059†	41

* Significantly less than that of initial plants.

† Highly significantly greater than corresponding values for initial plants.

TABLE 8

SUMMARY OF EXPERIMENTS ON INFLUENCE OF VARYING NIGHT TEMPERATURES ON GROWTH AND RUBBER FORMATION OF YOUNG GUAYULE PLANTS

Exp. no.	Length of exp. (months)	Temperature (°F.)		Change in rubber (%)	Increase in rubber per 2 plants (mg.)	Grams rubber formed per 100 gm. increase in stem and root dry wt.
		Day	Night			
G-25..............	2	80	80	−0.21†	23	0.32
G-39a.............	2	80	80	−0.15	67	0.39
G-39b.............	1	80	80	−0.08	5	0.08
G-42..............	1	80	80	−0.20†	− 4	0.00
G-43..............	1	80	80	−0.01	15	0.27
G-50..............	1	80	80	−0.07	23	0.37
G-52..............	1	80	80	−0.29‡	25	0.43
G-57..............	1	80	80	−0.22†	13	0.34
G-61..............	1	80	80	+0.11	12	0.64
G-62..............	1	80	80	+0.01	4	0.40
G-39a.............	2	80	60	−0.13	88	0.43
G-39b.............	1	80	55	+0.04	61	0.62
G-62..............	1	80	55	+0.11†	8	0.71
G-42..............	1	80	50	+0.19‡	54	0.88
G-43..............	1	80	50	+0.32‡	26	1.31
G-50..............	1	80	45	+0.99‡	126	2.57
G-52..............	1	80	45	+1.01‡	164	3.09
G-57..............	1	80	40	+0.66‡	127	2.72
G-61..............	1	80	40	+0.45‡	29	1.53

† Significant at 5% level.

‡ Significant at 1% level.

Table 8 gives for each experiment the percentage of rubber in the dry weight deposited during the experimental period. At night temperatures of 80°, 60°, and 55° this percentage is low, and of the order of the percentage rubber found in greenhouse, nursery, or field plants during the first summer (in the Institute field at Arcadia). With night temperatures of 50°, however, rubber constituted about 1% of the added dry weight; and with night temperatures of 45°, it constituted 2.6–3.1% of the total added dry weight. Thus, under the conditions used these young plants appear to be nearly ten times as efficient formers of rubber with nights at 45° as they are with nights at 80°. Many other factors affect this efficiency, however, as will be next shown.

CONTROLLED TEMPERATURES OVER 4-MONTH PERIOD.—The plants used for this experiment (G-52) were of the cull nursery stock. They were grown in gravel contained in 1-gallon cans. On September 28, 1942, plants selected for uniformity were transferred either to the greenhouse having a constant temperature of 80° or to the similar greenhouse having an 8-hour day at 80° combined with a 16-hour night at 45°. After 1 month, sample plants were taken from each condition (data used in table 6), and harvests were repeated after 2 and after 4 months. The data are given in table 9. Plants subjected to continuous temperature of 80° showed no significant change in rubber concentration over the 4-month period, although the absolute amount of rubber per plant increased 4.0 times. Plants subjected to the low night temperature increased steadily in rubber concentration and at the end of the 4-month period contained twelve times the amount present in the initial plants.

TABLE 9

GROWTH AND RUBBER FORMATION OF YOUNG GUAYULE PLANTS UNDER CONTROLLED TEMPERATURES. EXPERIMENT G-52. ANY COMPARISON ATTENDED BY 18° OF FREEDOM

MATERIAL	80° DAY AND NIGHT				80° DAY AND 45° NIGHT			
	Dry wt. per plant (gm.)		Resin (%)	Rubber (%)	Dry wt. per plant (gm.)		Resin (%)	Rubber (%)
	Stems and roots	Leaves			Stems and roots	Leaves		
Initial plants	2.81±0.216	3.41±0.215	4.39±0.091	1.00±0.112	2.81±0.261	3.41±0.215	4.39±0.091	1.00±0.112
1 month....	5.70± .444	7.26± .648	3.56± .092	0.71± .039	5.47± .509	5.63± .702	3.72± .092	2.01± .142
2 months...	6.38± .545	4.57± .347*	3.74± .102	1.04± .096	6.97± .775	4.30± .514	4.25± .080	3.21± .189
4 months...	13.06±0.607	11.92±0.714	3.65±0.089	0.85±0.069	7.60±0.60	5.34±0.412	4.59±0.087	4.18±0.138

* Reason for apparent decrease in leaf weight during this period not certainly known but may have been result of greenhouse fumigation.

Table 10 gives certain data derived from those of table 9, including the ratio of grams of rubber deposited to total dry weight deposited. This quantity, which was used in table 8, expresses—in a formal way at least—the efficiency of the plant as a rubber producer during the experimental period. In the plants maintained continuously at 80°, 0.46 gm. of rubber was laid down per 100 gm. new dry weight during the first month and 0.68 gm. of rubber per 100 gm. new dry weight during the last 2 months. During the second month, however, it was higher, owing to unrecognized reasons—perhaps related to the unknown cause of the leaf-drop during the second month. The plants which received nights at 45°

increased in rubber-forming efficiency markedly as the experiment progressed. During the last 2 months, 15 gm. of rubber was accumulated per 100 gm. of new dry weight, or five times as much as during the first month. During the last 2 months the plants maintained at 45° night temperature formed approximately twenty-two times as much rubber per 100 gm. new dry weight as did the plants maintained at 80°.

OLDER PLANTS.—Parallel to the experiment of table 9, another was run with older plants (G-51). The two tests were run simultaneously and are comparable in every way, except for the nature of the plants used. The plants were grown from 1940 nursery stock of the Intercontinental Rubber Company planted in the field in June, 1941. The soil of the field (gravel subsoil) appeared to be unsuitable, and the plants made little growth as compared with similar plantings in other locations. In March, 1942, the plants were transferred to gravel in 2-gallon crocks and grown outdoors until September 28. They presented an appearance of typical field-grown plants, although of smaller size. When the plants were taken from the field in March they contained 4.88% rubber, and this declined to 2.58% on September 28. Total rubber per plant increased during the same time. On September 28, 100 plants were selected for uniformity and distributed as in the experiment of table 9, a portion being maintained in the greenhouse at a constant temperature of 80° and a further portion maintained at a day temperature of 80° combined with a night temperature of 45°. Harvests were made after 1, 2, and 4 months. The first two harvests were based on ten individual plants from each condition, while the final harvest was based on ten lots of two plants each from each condition. The results are presented in table 11. Growth of the plants during the first month was marked under the constant 80° conditions. Abundant new growth appeared, and the plants flowered profusely, although no significant increase in total dry weight occurred (table 11). Throughout the 4-month period the plants which received 45° nights maintained a dormant aspect, made little or no new shoot growth, formed no new leaves, and did not flower. They did, however, increase appreciably in dry weight. The plants which received 80° nights produced new leaves and flowers throughout the 4-month period.

TABLE 10

DATA ON AMOUNTS OF RUBBER PER PLANT, ETC., DERIVED FROM TABLE 9

MATERIAL	80° DAY AND NIGHT		80° DAY AND 45° NIGHT	
	Rubber per plant (mg.)	Grams rubber formed per 100 gm. of total increase in wt.	Rubber per plant (mg.)	Grams rubber formed per 100 gm. of total increase in wt.
Initial plants	28	....	28	....
1 month....	40	0.43	110	3.1
2 months...	66	1.06	224	4.7
4 months...	111	0.81	318	6.0
2 months (last)....	....	0.67	....	14.9

With these plants, as with those of table 9, there was considerable difference in rubber formation between the two temperatures. Plants kept continuously at 80° did not change significantly in rubber concentration during the 4-month period, whereas those which received 45° nights increased steadily and more than doubled their original concentration. In fact, these latter plants had a final con-

centration as high as that of similar plants left outdoors during the same 4-month period (table 11).

It is not desired to stress the results of this experiment, since growth of the plants was relatively small during the experimental period (table 11). Growth of the same plants was rapid during the summer, and the subsequent lesser growth may have been due to a pot-bound condition. The conclusions drawn are therefore strictly applicable only to these particular plants.

TABLE 11

GROWTH AND RUBBER FORMATION OF OLDER GUAYULE PLANTS UNDER CONTROLLED TEMPERATURES. EXPERIMENT G-51. ANY COMPARISON ATTENDED BY 18° OF FREEDOM

MATERIAL	80° DAY AND 80° NIGHT				80° DAY AND 45° NIGHT			
	Dry wt. per plant (gm.)		Resin (%)	Rubber (%)	Dry wt. per plant (gm.)		Resin (%)	Rubber (%)
	Stems and roots	Leaves			Stems and roots	Leaves		
Initial plants	72.2±4.73	42.8±1.91	4.49±0.093	2.58±0.233	72.2±4.73	42.8±1.91	4.49±0.093	2.58±0.233
1 month	69.8±3.46	48.3±1.72	4.03± .084	2.38± .157	80.6±4.58	41.9±1.73	4.71± .177	3.21± .281
2 months	81.9±4.27	54.2±2.56	4.22± .145	2.56± .167	95.5±3.52	45.2±1.50	4.84± .092	4.18± .211
4 months	83.6±2.56	46.2±2.50	4.37±0.063	2.67±0.195	90.2±3.00	37.2±1.50	4.94± .116	5.75± .107
4 months outdoors*	....	....	....	....	78.5±4.06	12.7±0.83	5.42±0.103	5.50±0.212

* Many leaves dislodged by heavy rain.

TABLE 12

DATA ON AMOUNTS OF RUBBER PER PLANT, ETC., DERIVED FROM TABLE 11

MATERIAL	80° DAY AND 80° NIGHT		80° DAY AND 45° NIGHT	
	Rubber per plant (gm.)	Grams rubber formed per 100 gm. total increase in dry wt.	Rubber per plant (gm.)	Grams rubber formed per 100 gm. total increase in dry wt.
Initial plants	1.86	....	1.86	....
1 month	1.67	....	2.58	8.7
2 months	2.09	2.37	3.99	9.1
4 months	2.23	3.24	5.20	18.5

Table 12 presents data derived from table 11 as to total rubber per plant and as to rubber deposited per unit of added dry weight. Comparison of tables 10 and 12 shows that: night temperature of 45° induced more rubber formation than night temperature of 80°, both with young and with older plants; with both kinds of plants, response to the lower night temperature was more striking over a 4-month than over a 1-month period; and under both temperature conditions the older plants were markedly more efficient rubber producers than the young ones.

It would appear from the preceding experiments that night temperatures may be of importance in regulating rubber formation in guayule. That other factors, such as nutritional status, water supply, etc., also influence rubber accumulation may be assumed. The relation of these several factors and the relative significance of each are matters for further investigation.

Summary

1. Young greenhouse-grown plants of guayule showed marked difference in rubber formation according to the temperature conditions to which the plants were subjected.

2. Plants grown in the greenhouse or outdoors in the summer contained in general 0.50% or less rubber. When such

plants were transferred to a greenhouse at a constant temperature of 80° F., rubber concentration either remained at the initial low level or dropped slightly.

3. Similar plants subjected to an 8-hour day at 80° and a 16-hour night at 45° or 40° increased in percentage of rubber. This increase amounted to 0.7–1.0% over a period of 1 month.

4. Night temperatures of 50° appeared to be less effective than night temperatures of 40°–45° in causing increase of rubber percentage. Night temperatures of 55°–60° were ineffective.

5. Young plants continued to respond to night temperatures of 45° with increased rubber production over a period of 4 months.

6. Older plants, which had already been subjected to one winter in the field, responded to low night temperatures in a manner qualitatively similar to that of young plants. The older plants, however, were more effective in rubber accumulation than the young plants, both at high and at low night temperatures.

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NATURE AND RATE OF DEVELOPMENT OF ROOT SYSTEM OF LEPIDIUM DRABA[1]

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Introduction

A series of studies of growth habits of noxious perennial weeds of central United States was undertaken at Manhattan, Kansas, in 1937 by the Kansas Agricultural Experiment Station. A study (1) of the nature and rate of development of the root system of field bindweed established the scope and methods of procedure. The second of these investigations concerns *Lepidium draba* L.,[2] to which several common names have been applied, including hoary cress, whitetop, whiteweed, and perennial peppergrass. It is a noxious weed of potentially equal or greater significance than field bindweed in this area.

Environmental conditions and methods

Soil data.—All plants[3] considered in this paper were taken from soil, the nature of which is described in the following profile description supplied by Dr. J. C. Hide of Kansas Agricultural Experiment Station:

Depth 0–8 inches.—A dark grayish brown silt loam to silty clay loam with weakly developed platy structure, except in the lower 2 inches which has a slightly granular nature.

Depth 8–14 inches.—A dark brown loamy clay; moderately compact. Angular cleavage with only slight, if any, tendency to columnar structure. Aggregates relatively fine, about $\frac{1}{8}$ inch. A few small limestone fragments.

Depth 14–22 inches.—A dark yellowish brown loamy clay with fairly well-developed fragmental to blocky structure. Little tendency to form columns. Moderately friable.

Depth 22–60 inches.—A yellowish brown loamy clay. Blocky aggregates show little tendency to form columns. Small fragments of limestone abundant but less numerous below 36–38 inches. Below 32 inches, lime has been deposited in root channels.

The soil was a fairly typical Geary silt loam, except that it lacked the reddish brown color distinctive of that soil type. No layer of this profile appears to be of such nature as to impede seriously the development of the roots of deep-rooting plants.

Meteorological data.—The monthly and annual temperature and precipitation for the first 10 months of 1941 compared with the long-time average of these two factors are given in table 1. The summer of 1941 had temperatures of 100° F. or higher for 15 days, as compared with 38 such days in 1937 and 60 days in 1936. The average number of days at this temperature for the 50 years (1889–1938) is 15. The monthly and annual mean temperatures were higher than those for the 85-year period, 1856–1940, except for the monthly means of March and June. The average monthly precipitation for the first 10 months of 1941 was 3.22 inches, as compared with the monthly average 2.86 for these months during the 83-year period (1858–1940).

Methods.—The plants were grown on a small plot of land free of both noxious

[1] Contribution no. 451, Department of Botany, Kansas Agricultural Experiment Station.

[2] The great variations in the degree of veining and in the shape of the capsule within our individual plants correspond to the findings of Hitchcock (2); hence varieties under the species are not recognized.

[3] The term plant as used in this paper refers to all the growth from a single seed. The term shoot is applied to the individual leafy stems commonly called plants in control studies.

weeds and sodium chlorate. It adjoined that described previously (1). The soil is a fairly typical Geary silt loam representative of the wind-deposited soil of the upland of this locality. The plot had been cultivated a decade earlier but had not been disturbed recently.

Plantings were made on April 21, 1941. Twenty-five seeds, which had been kept moist at 34° F. for 41 days, were placed $\frac{5}{16}$ inch below the surface at each of twenty-two points which were so spaced that seven of the plantings were 14 feet distant from any other, and the remainder were separated by 5–9 feet. Emergence of seedlings began on April 26, and on May 1 at least one seedling emerged at eighteen of the twenty-two points. All previously emerging seedlings were removed, as were those emerging after this date; consequently, all plants considered in this study emerged on May 1. The area was kept free of other vegetation, so that the only competition for water and nutrients, if any existed, was among the plants of hoary cress.

Root systems were excavated at weekly intervals for the first 7 weeks, and also 9, 12, 26, and 75 weeks after emergence, by modification of the trenching method developed by WEAVER (6) as described by FRAZIER (1).

Effort was made to keep the root system as one entity; if one broke, the two ends were immediately tied together. Measurements of the plant parts were made and recorded, and the relationship of parts noted as the root system was excavated, so that the plant as illustrated occupied the same relative relationship one part to another as it did in the soil, except that in the figures all lateral roots are arranged in one plane. While as many of the finer roots were obtained as possible, it is not contended that a large proportion was secured.

Observations

The rate of plant development under known soil and climatic conditions was observed by means of seven plants excavated weekly between May 8 and June 19, and plants 8, 9, and 10 excavated July 3, 24, and in late October, 1941, respectively. An eleventh plant was excavated in mid-October, 1942. Figures 1–5, inclusive, show the vertical penetration and figures 3–5 the radial spread of representative plants. Data of the eleven excavations are given in table 2.

TABLE 1

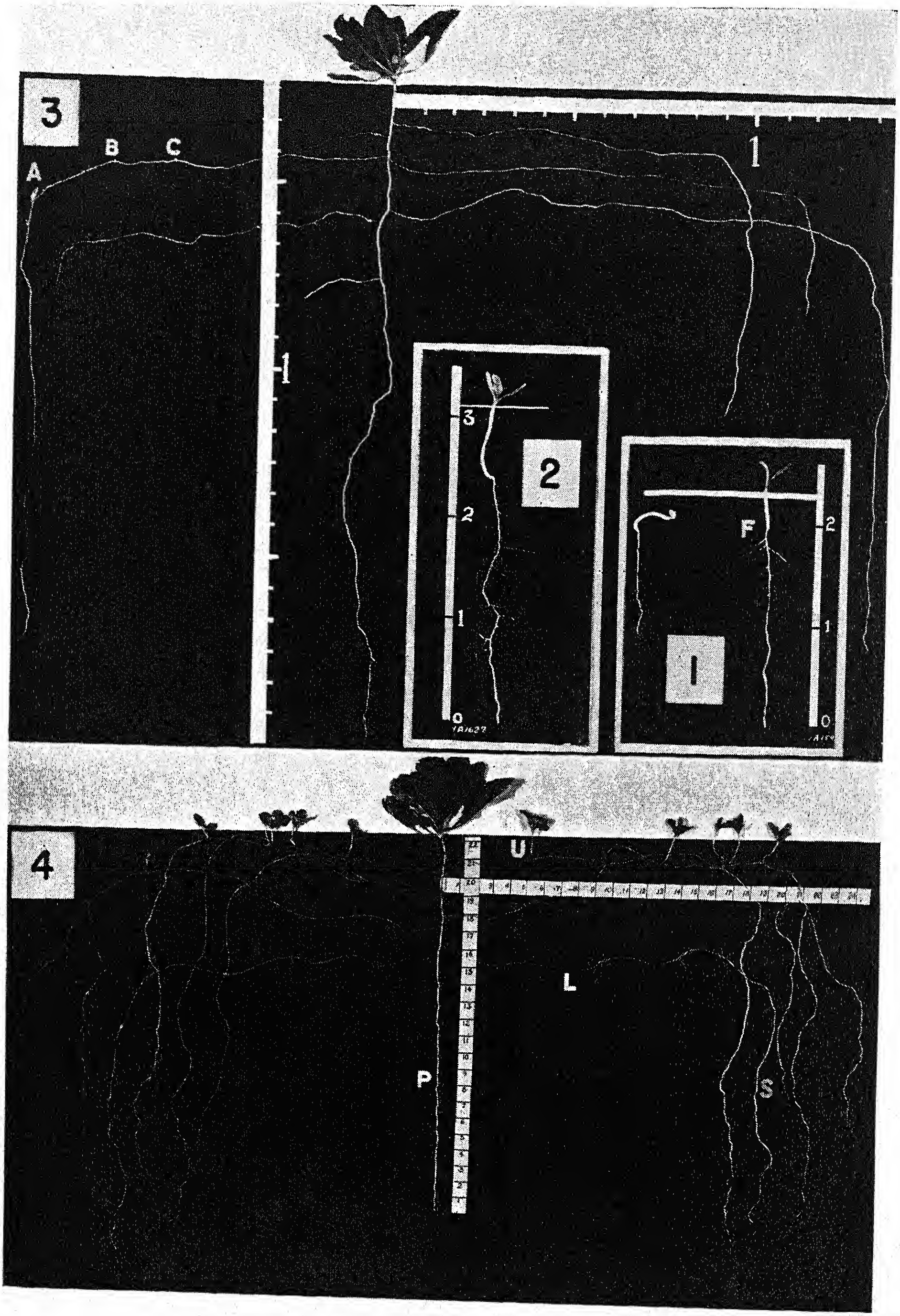
MONTHLY AND ANNUAL TEMPERATURES (IN °F.) AND PRECIPITATION (IN INCHES) AT MANHATTAN, KANSAS, 1941

MONTH	TEMPERATURE		PRECIPITATION	
	Mean*	85-year average (1856–1940)†	Total*	83-year average (1858–1940)†
Jan.	32.2	27.0	2.08	0.75
Feb.	33.1	31.5	0.28	1.14
Mar.	40.6	42.5	1.23	1.48
Apr.	58.5	54.5	1.81	2.67
May	69.6	64.6	2.36	4.31
June	74.0	74.5	3.80	4.50
July	81.4	79.5	1.29	4.25
Aug.	79.8	77.5	3.00	3.89
Sept.	71.9	69.3	4.85	3.44
Oct.	59.2	56.5	11.50	2.16
Annual mean or total	57.0	54.2	33.93	30.97

* Meteorological data obtained from U.S. Weather Bureau, Kansas Section.

† Computed by Dr. A. B. CARDWELL of Kansas State College.

By the seventh week after emergence, the general plan of the root system was beginning to be established. The plant taken the twelfth week after emergence (fig. 4) showed the gross morphological nature of the root system particularly well. A taproot rapidly penetrated directly down from the germinating seed.



FIGS. 1–4.—Development of plants of hoary cress. Fig. 1, seedling at left had not emerged, seedling at right one week after emergence; *F*, root-stem transition zone. Fig. 2, seedling 2 weeks after emergence showing first true leaves. Fig. 3, young plant 9 weeks after emergence: *B*, *C*, root-borne stem buds; *A*, bud giving rise to rhizome; secondary vertical roots have grown downward 15–16 inches. Fig. 4, complete plant 12 weeks after emergence (not all primary vertical root obtained): *P*, primary vertical root; *L*, lateral root of first order; *S*, secondary vertical of first order; *U*, rhizome arising from lateral root. Figs. 1, 2, 4, scale in inches; fig. 3, scale in feet and inches.

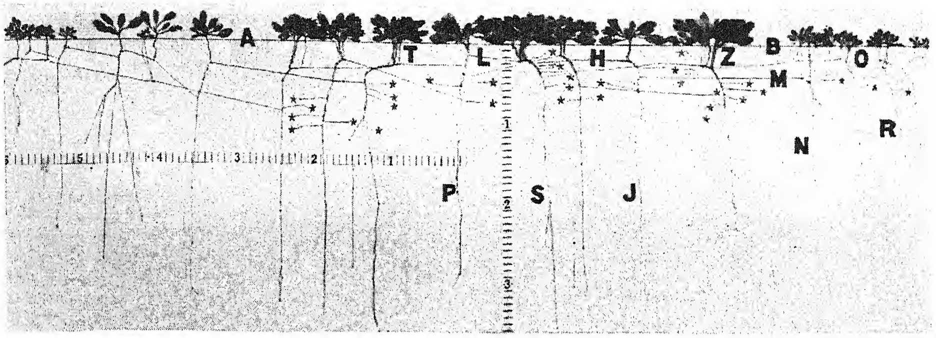


FIG. 5.—Approximately 60% of growth attained by one plant of hoary cress $6\frac{1}{2}$ months after emergence, arranged to show place relationship of parts essentially as they were in the soil. Stars indicate points from which lateral roots have been removed for clarity. *A–B* is ground line. Lateral roots to left of primary vertical developed under sloping ground line, which causes them to appear to grow upward from point of origin when arranged to a horizontal ground line. *P*, primary vertical root; *L*, permanent lateral of first order; *S*, secondary vertical of first order; *H*, permanent lateral of second order; *J*, secondary vertical of second order; *M*, permanent lateral of third order; *N*, secondary vertical of third order; *O*, permanent lateral of fourth order; *R*, secondary vertical of fourth order. Scale in feet and inches.

TABLE 2

RATE AND NATURE OF DEVELOPMENT,* AFTER SEEDLING EMERGENCE, OF ROOTS OF HOARY CRESS PLANTS

Weeks after emergence	Maximum vertical penetration (inches)	Maximum radial spread (inches)	Plant condition
1	$2\frac{1}{4}$	....	Long, somewhat linear cotyledons (fig. 1)
2	$3\frac{1}{8}$	$1\frac{1}{8}$	First true leaves (fig. 2)
3	$6\frac{3}{4}$	2	Additional true leaves
4	$10\frac{3}{4}$	$3\frac{7}{8}$	Older leaves becoming characteristically shaped
5	$14\frac{3}{4}$	$6\frac{1}{4}$	Several new leaves; forming loose rosette
6	$18\frac{1}{8}$	10	Cotyledons lost
7	$19\frac{1}{2}$	$15\frac{3}{4}$	Lateral roots growing downward at tip; first root-borne stem buds on lateral roots, 13–14 inches from primary vertical
9	$21\frac{1}{4}$	$16\frac{1}{2}$	Well-developed root-borne stem buds on primary permanent lateral and near juncture of that root and secondary vertical roots, one of which is developing into a rhizome; vertical roots have grown downward 15–16 inches (fig. 3)
12	22+	$24\frac{1}{2}$	Root-borne stem buds have formed rhizomes, most of which have emerged and developed shoots. Not all of primary vertical root excavated (fig. 4)
26	37	$73\frac{1}{2}$	By now the root development included permanent lateral and secondary verticals through fifth order (fig. 5)
75 (1942)	$11\frac{1}{4}$ ft.	At least 10–12 ft.	Secondary vertical roots apparently penetrated deeper than primary verticals and were larger in diameter, particularly in first 12–24 inches below bend where lateral root becomes secondary vertical

* First flowering of these plantings occurred June 18, 1942.

It was a primary root in order of development and vertical in position; hence, designated the primary vertical root (fig. 4*P*). Many branch roots arose throughout the length of this taproot, most of them small feeding roots. A few, probably those more favorably located, grew extensively and became permanent parts of the root system. They are designated permanent lateral roots (fig. 4*L*). These grew horizontally 12–26 inches and then turned down in a broad sweeping bend and were from that point vertical taproots, designated secondary vertical roots[4] (fig. 4*S*). The permanent lateral roots of any order, which are usually smallest in diameter at their origin, were always in the surface foot, more commonly in the upper 7 inches of the soil. The lateral-vertical roots of the first order gave rise to branch roots over any part of the horizontal or vertical extent. Most of these were small feeding roots. However, at or just below the bend where the primary permanent laterals become secondary vertical roots, permanent laterals of the second order developed (fig. 5*H*). These extended horizontally 7–20 inches from the point of origin, the general direction of growth being away from the primary vertical root, and turned down to form secondary vertical roots of the second order (fig. 5*J*). This development was repeated many times during the growing season to give rise to lateral-verticals of the third, fourth, and even higher orders. It is by such series of permanent lateral roots that horizontal spreading of the plant is accomplished. If more than one permanent lateral arose from the place of origin of such roots of the second or higher order, they radiated from the point of origin; but, in general, all maintained growth away from the primary vertical root. The result was that the plant occupied a higher proportion of the ever-increasing area, somewhat circular in shape, than it would have otherwise.

All shoot development, except that from the plumule, was derived from root-borne stem buds. These developed on any part of the permanent root system (that is, the permanent laterals and the two types of vertical roots). Twelve weeks after emergence, a number of these buds had been formed on or near the juncture between the permanent lateral and the secondary vertical roots, as well as a few along the permanent lateral roots themselves, and most of these had produced rhizomes which on emergence had produced shoots (fig. 4). The location of most extensive shoot development from root-borne stem buds was at or near the bend between the lateral root of any order and its vertical phase (fig. 5*T*, *Z*). Infrequently, shoots not subtended by a vertical root were found along the permanent laterals (fig. 4*U*). Regardless of where these root-borne stem buds formed, they gave rise to rhizomes unless the buds were borne at the ground line, in which case they gave rise to leafy shoots.

Fourteen weeks later, or 26 weeks after the plant had emerged as a seedling, the primary vertical root had penetrated 37 inches vertically and had given rise to nine horizontal roots which were arranged radially around the primary vertical root. All nine of these permanent laterals had developed secondary vertical roots. Seven of the secondary verticals of the first order had given rise to lateral-secondary vertical roots of the second and higher orders. There were seventeen regions of shoot development on the nine permanent lateral roots. The

[4] The horizontal and vertical phases of these roots are included in the term lateral-vertical root. The vertical phase is considered to begin where the slender lateral root enlarges markedly.

shoot growth of these seventeen regions came from forty-one rhizomes, each of which arose from a root-borne stem bud. The growth attained 26 weeks after emergence was so carefully removed that practically all the plant was recovered. Approximately 60% of the growth constituting the plant was arranged to give the place relationship of these parts essentially as they were in the soil (fig. 5).

Discussion

The rate of growth of plants of hoary cress from seed under known soil and climatic conditions, with little or no competition, is not to be construed as representing their growth rate under all conditions. It illustrates only the growth potential under this favorable noncompetitive situation.

SIMONDS (5) reported no structural differences between the horizontal and perpendicular roots of this plant and found that both produce adventitious buds which develop into shoots.

No reason was determined why certain of the branch roots develop more extensively to become permanent lateral roots. It appears probable that this is a result of their more favorable location. KENNEDY and CRAFTS (3) gave this explanation for the development of permanent lateral roots in field bindweed.

The observations of PEITERSEN and BURDICK (4) and SIMONDS (5) on the general nature of the vertical and horizontal portions of the root system of hoary cress agree with the findings of this study. The former designated the horizontal roots as root stocks, which is misleading, in that this term is commonly used for rhizomes (underground stems).

Summary

1. Hoary cress plants, grown from seed on a typical upland loam soil at Manhattan, Kansas, under known temperature and precipitation conditions, and not subject to competition, were studied at various ages, from the seedling stage through 75 weeks of growth, to determine the nature and rate of development.

2. The root system consisted of the original root (primary vertical) and one to many permanent laterals, the vertical extensions of which were designated secondary vertical roots.

3. The plants spread horizontally by means of series of permanent lateral roots. The primary permanent laterals arise on the primary vertical root. Unless injury or too severe competition prevents, successive orders of permanent laterals arise at or near the bend where a permanent lateral of the preceding order turns down to become a vertical root.

4. The plants spread radially $6\frac{1}{8}$ feet in one growing season and $11\frac{1}{4}$ feet by the end of the second season, at which time many verticals had reached a depth of 10–12 feet.

5. The source of shoot development, other than that arising from the plumule, was from root-borne buds which form either leafy shoots directly (if at the ground line) or rhizomes (if below ground) which in turn give rise to leafy shoots. These arose in greatest abundance at or near the bend separating the lateral root of any order from its vertical phase. The shoot development of old plants is wholly from root-borne buds.

6. The type of development is similar to that of field bindweed. The rate of development of hoary cress does not appear to be as rapid as that of field bindweed during the first 75 weeks of growth.

DEPARTMENT OF BOTANY
KANSAS AGRICULTURAL EXPERIMENT STATION
MANHATTAN, KANSAS

LITERATURE CITED

1. FRAZIER, J. C., Nature and rate of development of root system of *Convolvulus arvensis*. BOT. GAZ. 104:417–425. 1943.
2. HITCHCOCK, C. L., The genus *Lepidium* in the United States. Madroño 3:265–320. 1936.
3. KENNEDY, P. B., and CRAFTS, A. S., The anatomy of *Convolvulus arvensis*, wild morning-glory or field bindwind. Hilgardia 5:591–622. 1931.
4. PEITERSEN, A. K., and BURDICK, R. T., Perennial peppergrass—a noxious weed in Colorado. Colorado Exp. Sta. Bull. 264. 1920.
5. SIMONDS, A. O., The anatomical development of *Lepidium draba*. Jour. Agr. Res. 57:917–928. 1938.
6. WEAVER, J. E., Root development of field crops, McGraw-Hill, New York. 1926.

GERMINATION OF THE NUTS OF THE TUNG TREE AS AFFECTED BY PENETRANTS, SUBSTRATA, DEPTH OF PLANTING AND STORAGE CONDITIONS

C. B. SHEAR[1] AND H. L. CRANE[2]

Introduction

A method which would materially shorten the period required for and produce more uniform germination of tung (*Aleurites fordii* Hemsl.) nuts would alleviate difficulties experienced in nursery practice. The accepted practice is to plant the nuts in February or March. About 60 days are required for germination, but many seeds do not germinate until the following year. Emergence occurs when there is likelihood of injury to the seedlings by high temperatures. Quick, uniform germination of the seeds would produce trees of less variable size and suitable for budding earlier in the summer than normally is possible with present methods.

SHARPE and MERRILL[3] found that seeds that were hulled and stratified consistently germinated earlier than those subjected to other treatment, apparently mainly due to the intake of water during stratification.

At the time the present experiments were planned, the beneficial effects of stratification had not been reported, but under aseptic conditions the kernels of shelled nuts showed that initiation of germination was possible within 5 days. This indicated that any delay was due to inability of the seeds readily to absorb water through the thin but hard shell of the nut. Experiments were planned to determine: (*a*) whether it was possible to increase the rate of water absorption through the use of surface-tension-reducing chemicals; (*b*) the effect on germination of various substrata differing in their water-holding and aeration capacities; (*c*) the effect of planting depths; and (*d*) the effect of various periods of storage at different temperatures.

Experimental results

EFFECT OF PENETRANTS

The penetrants used were morpholine and tergetol, both products of the Union Carbide and Carbon Corporation of New York. To test their effect on germination, lots of 100 open-pollinated seeds from a single tree, after careful cleansing, were treated as follows: morpholine 0.25, 0.5, and 1.0% for 12, 24, and 48 hours; tergetol 0.1, 0.2, and 0.4% for 12, 24, and 48 hours; water for 12, 24, and 48 hours; and untreated.

The nuts were placed in glazed crocks and covered with the solutions of morpholine or tergetol, or with water, at such times that the 12-, 24-, and 48-hour treatments would all be ready for removal and planting at the same time. The temperatures of the solutions and water varied from 24° to 27° C. Upon removal from the solutions, the lots of seeds were rinsed with tap water and planted in the greenhouse in a bed of well-rotted sawdust, the treatments being arranged in randomized replications of twenty seeds each. The planting was made on March 13, 1941; and emergence counts were made daily, with a few exceptions, after April 1—when the first seedlings appeared.

MORPHOLINE.—The data for cumulative emergence on successive days after

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[3] SHARPE, R. H., and MERRILL, SAMUEL, JR., Effects of stratification and time of planting on germination of tung seed. Proc. Amer. Soc. Hort. Sci. 40: 286–291. 1942.

planting for the 48-hour treatments are graphed in figure 1. Chi square values for a number of pertinent comparisons, also for the 48-hour treatments, are presented in table 1. A marked increase in rate of emergence over unsoaked seeds

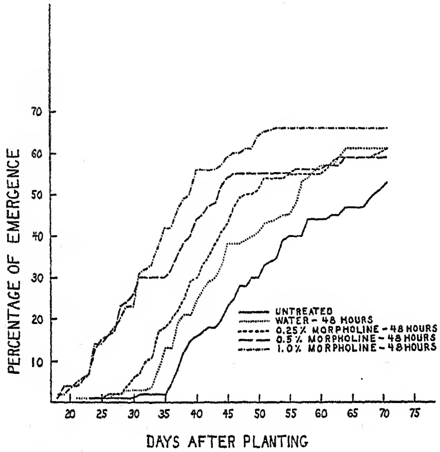

FIG. 1.—Effect of treatment with water and morpholine at different concentrations on percentage of emergence of tung seedlings.

resulted when the seeds were soaked in water for 48 hours; similar increases resulted from soaking for 12 or 24 hours. Morpholine at a concentration of 0.25% for 48 hours increased the rate significantly over that obtained with the same period of treatment with water, but after 12 or 24 hours at this concentration the rate of emergence was not significantly increased over that resulting from an equal period of soaking in water. When the concentration was increased to 0.5%, treatment for 12 hours was as effective as was the 48-hour treatment in the 0.25% solution. Soaking for 24 hours significantly increased the rate over the 12-hour treatment, and the 48-hour treatment was significantly increased over the 24-hour one.

Increasing the strength of the solution to 1.0% and soaking for 12 hours showed no advantage over the 0.5% treatment for the same period, but at 1.0% for 24 hours the results were equivalent to those obtained at 0.5% for 48 hours. However, when a 1.0% solution was used for 48 hours, the rate of emergence was increased significantly over that resulting from treatment with the same concentration for 24 hours. Comparison of the curves for these treatments in figure 1 shows that soaking the seed in 1.0% morpholine for 48 hours resulted in comparable percentages of emergence 12–18 days ahead of seed soaked for the same period in water, and 16–30 days ahead of untreated seed. There was no indication of any toxic effect from any of the morpholine treatments used.

There is also a definite tendency for decrease in rate of emergence to be associated with decrease in the final percentage of germination. At the termination of the experiment, the oil in the endosperm of many of the ungerminated seeds had solidified. This condition is

TABLE 1

CHI SQUARE VALUES FOR COMPARISONS OF RATES OF EMERGENCE OF TUNG SEEDLINGS

Contrast of treatments (each 48 hours)	df	Chi square*
Untreated vs. H_2O	42	8.6
Untreated vs. 0.25% morpholine	40	49.6
Untreated vs. 0.5% morpholine	42	279.8
Untreated vs. 1.0% morpholine	42	226.3
H_2O vs. 0.25% morpholine	40	18.8
H_2O vs. 0.5% morpholine	43	167.5
H_2O vs. 1.0% morpholine	43	162.7

* Goulden, C. H., Methods of statistical analysis. John Wiley and Sons, New York. 1939.

probably an important factor in decreased viability of seed whose germination has been delayed.

TERGETOL.—All the tergetol treatments, with the exception of 0.1% for 12 and for 24 hours, were toxic to such an

extent that germination was reduced to 0–10%. Tergetol at a concentration of 0.1% for 24 hours was as effective in hastening emergence as was the 48-hour treatment with 0.5% morpholine, indicating the former a much more powerful penetrant. However, the beneficial effects obtained with tergetol are of doubtful value in practical application, because of the risk of toxic effects.

As the concentration of morpholine increased, the rate of emergence was not in proportion to the increase in moisture content of the kernel, which was approximately the same in seeds treated with 1.0% morpholine as in those treated with 0.5% for the same periods. The rate of emergence of the former was markedly higher than that of the latter, indicating that morpholine has some effect on germination beyond the increase in moisture content at the time of planting. There are two possible explanations for this effect: (*a*) At the higher concentration, morpholine, owing to its marked solvent properties, may increase the permeability of the shell to such an extent that, after planting, the rate of supply of water and of oxygen to the germinating embryo is greater than for seed treated with lower concentrations. (*b*) Morpholine, structurally related to ethylene, which has been found to stimulate proteolytic enzyme activity, may stimulate germination in this or some other way.

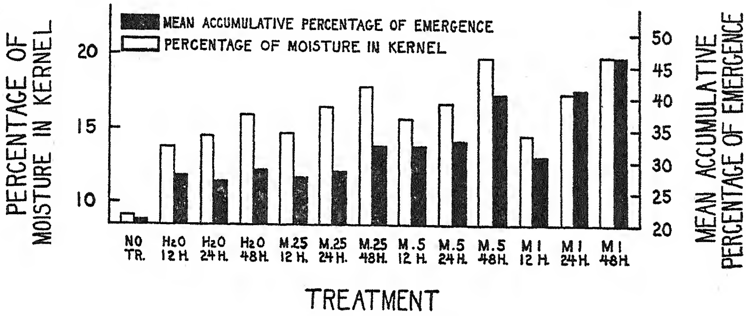

FIG. 2.—Relation of percentage of moisture in kernel at time of planting to mean cumulative percentage of emergence of seedlings. Mean cumulative percentage of emergence obtained by dividing the total percentage of emergence from the eighteenth to the seventy-first day after planting by the number of observations included in this total.

EFFECT OF MOISTURE CONTENT OF KERNELS

Samples of twenty nuts each, in duplicate, were treated with morpholine in the same concentrations and for the same periods as those used in the germination tests. After soaking, the shells were removed, the weights of the kernels obtained, and they were then dried for 20 hours at 100° C., again weighed, and moisture percentages calculated.

In figure 2 the percentage of moisture in the kernels is plotted against the mean cumulative percentage of emergence for the same treatments. There is a positive correlation between the rate of emergence and the moisture content of the kernel at the time of planting. The coefficient of correlation between these two measurements is 0.869, with a standard error of 0.0738.

EFFECT OF VARYING SUBSTRATA

It has been our practice to use well-rotted sawdust as a substratum for germinating seed. In 1942 it was necessary to use fresh sawdust—containing con-

siderable soil—for part of the planting, as the source of rotted sawdust had been depleted. The rate and final percentage of germination in the fresh sawdust were so superior that an experiment was set up to determine the effects of different substrata on germination.

Six media, varying widely in respect to air- and water-holding capacities, were selected: (*a*) sphagnum moss; (*b*) Berkeley Springs "F" grade glass sand; (*c*)

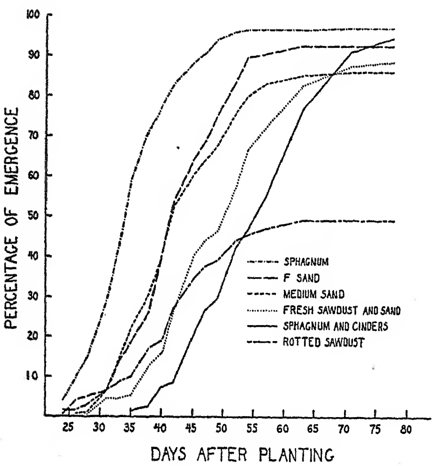

Fig. 3.—Effect of substrata on percentage of emergence of seedlings.

Berkeley Springs medium-grade glass sand; (*d*) a mixture of 64 parts by weight of fresh sawdust plus 36 parts of a one-to-one mixture of the medium and 8-mesh glass sand; (*e*) a mixture of 16 parts by weight of cinders screened through a 4-mesh screen plus one part of screened sphagnum moss; and (*f*) well-rotted sawdust.

A lot of 200 open-pollinated seeds from one tree was planted at a depth of $\frac{3}{4}$ inch in each of these substrata, all seeds having previously been treated with 1.0% morpholine for 48 hours. The planting was made on January 30, 1942, and emergence counts were taken at 2- to 3-day intervals after February 23, when the first seedlings appeared.

Interpretation of the data for cumulative emergence in the different substrata (fig. 3) is complicated by the fact that the bench in which the experiment was planted abutted the west wall of the greenhouse, thus subjecting the sphagnum and cinder treatment to lower temperatures than the others. This condition probably contributed to the delayed germination of this treatment, and this temperature effect may have influenced to a lesser extent the germination in the fresh sawdust and sand treatment, which was second from the wall. Reliable evaluation of the effects of this condition being impossible, it cannot be considered in the comparison of results.

The outstanding features of the data in figure 3 are the results in the sphagnum moss. Both rate and final percentage of emergence were greatly increased in this medium over those in the other media, the chi square value for the comparison of the rate of emergence in this substratum with that resulting in rotted sawdust being 101.5, with 17 degrees of freedom. The fresh sawdust and sand mixture produced significantly better results than did the rotted sawdust or the sphagnum and cinder mixture, and both sands proved to be even more favorable media, the F sand showing a slight advantage over the medium sand.

Effect of planting depth

Seed was taken from the same lot as that used in the substratum experiment, treated with 1.0% morpholine for 48 hours, and planted in fresh sawdust at three depths—$\frac{1}{2}$, 1, and 2 inches. Each treatment consisted of five replications of twenty seeds each. Planting was done on January 30, 1942, and emergence counts were taken at 2- to 3-day intervals

after the first seedlings appeared, on March 6.

At a depth of $\frac{1}{2}$ inch, emergence commenced (fig. 4) at about the same rate as at 1 inch, but after 2 weeks this rate began to fall off, the final percentage of emergence attained by this lot being only 69% as compared with 93% for the seed planted at a depth of 1 inch. When seed was planted 2 inches deep the rate of emergence was greatly retarded, and the final percentage was 47%.

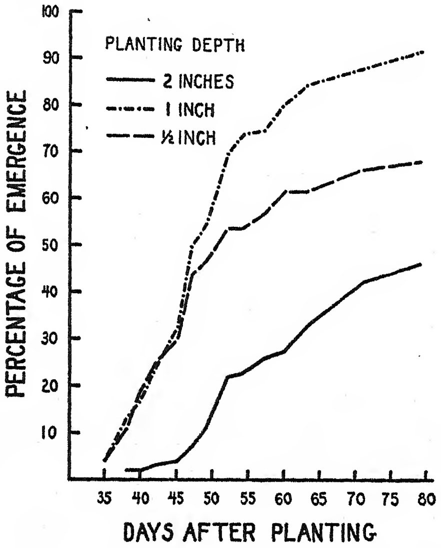

FIG. 4.—Effect of depth of planting on percentage of emergence of seedlings.

EFFECT OF STORAGE TEMPERATURE

The remainder of the seed from which the samples used in the morpholine and tergetol treatments were taken was divided into three lots, each being placed in storage at different temperatures on March 11, 1941. Prior to this date this seed had been stored (from December, 1940, to the first week of March, 1941) in the loft of a garage at the U.S. Field Laboratory for Tung Investigations, Bogalusa, Louisiana. One of the three lots was stored in a refrigerated room kept at approximately 7° C. The second lot was kept in the laboratory, where the temperature during this period was 23°–32° C. The third lot was stored in the attic of the laboratory building at Beltsville, where the temperature varied from

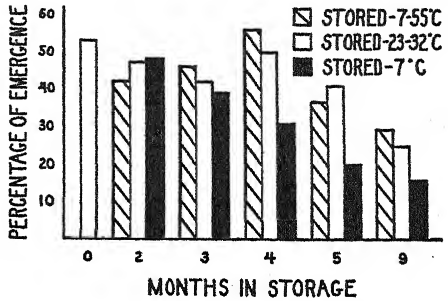

FIG. 5.—Effect of storage temperature and length of storage on percentage of emergence of seedlings.

about 7° to 55° C. Samples of 100 seeds were removed from each of these lots after storage for 2, 3, 4, 5, and 9 months and planted in rotted sawdust in a greenhouse bench. Planting was done in five randomized replications of twenty seeds each.

After 2 months' storage there was a decrease in the viability in all treatments (fig. 5), the greatest drop being in the sample taken from the lot stored at the highest temperatures. At 3 months, germination continued to decrease in the seed from the refrigerator and laboratory but increased in that from the attic. After 4 months in storage, both the lot from the attic and that from the laboratory showed increased germination. The refrigerated seed continued to lose viability, however, but that from the attic and laboratory continued to maintain an advantage over that from the refrigerator.

Discussion

The data presented in this paper substantiate the theory that the delayed germination of tung nuts under ordinary planting conditions is due primarily to

their slow rate of water absorption. They also corroborate the finding of SHARPE and MERRILL that the greater water intake during the period of stratification is responsible for the beneficial effect of this treatment.

The results of the substrata and depth-of-planting experiments not only further emphasize the importance of adequate water supply in hastening germination, but also indicate that, even when the moisture content of the seed is high at the time of planting and planting is done in a substratum of high water-holding capacity, some other factor has an important bearing on the rate and final percentage of germination. The indications are that this factor is aeration.

Although determinations of the relative water- and air-holding capacities of all the different media were not made, there are outstanding differences as regards these characteristics which are easily recognizable. Sphagnum, the substratum in which best results were obtained, undoubtedly has the greatest water-holding capacity of all the media used; at the same time it probably presents the most favorable conditions of aeration.

Determinations made in connection with the use of medium sand and F sand in nutritional experiments indicate that the F sand has only one-half the water-holding capacity of the medium sand but has sixteen times the air-holding capacity. Both of these media would be more subject to drying than the sphagnum, but frequent watering during the course of the experiment prevented moisture from becoming a critical factor in these substrata. The slight difference between the rates of germination in these two media is in favor of the coarser sand.

Even the addition of sand to the fresh sawdust evidently did not increase the porosity enough to supply adequate aeration for optimum germination, and the same condition was probably responsible for the slow rate of emergence in the screened sphagnum and cinder mixture.

The very slow rate of emergence and low final percentage of germination in the rotted sawdust undoubtedly are due to the tendency of this medium to pack and practically exclude air.

In the depth-of-planting experiment, the poor results obtained at a depth of $\frac{1}{2}$ inch are probably due to drying of the seed between applications of water, but the drop in rate and final percentage of germination resulting from planting at a depth of 2 inches seems to be further evidence of the detrimental effects of lack of aeration.

Summary

1. Morpholine was highly effective in increasing the rate of germination of tung nuts. Soaking in 1.0% for 48 hours attained practically the same percentage of emergence 12–18 days prior to that of nuts soaked for the same period in water, and they were 16–30 days ahead of untreated seed.

2. Seed planted in sphagnum reached the same percentage level of seedling emergence 29 days ahead of that planted in rotted sawdust, 21 days ahead of that planted in a mixture of sphagnum and cinders, 16 days ahead of that planted in a mixture of fresh sawdust and sand, and 7 days ahead of that planted in F sand or medium sand.

3. Seed planted at a depth of 1 inch germinated much more rapidly and attained a higher final percentage of germination than did seed planted at depths of $\frac{1}{2}$ inch or 2 inches.

4. Seed stored at 7° C. lost its viability sooner than did seed from storage that ranged either from 23°–32° or 7°–55° C.

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FRUIT SHAPE OF WATERMELON AS AFFECTED BY PLACEMENT OF POLLEN ON STIGMA

LOUIS K. MANN[1]

Introduction

Misshapen fruits in commercial watermelon plantings sometimes constitute a significant loss. Although the exact causes of asymmetry are not known, genetic factors, temperature or moisture gradients, mechanical injury, and other agents may be involved. In most plants the set and development of fruit is controlled somewhat by natural growth-regulating substances, whose activity and distribution are closely associated with pollination, growth of pollen tubes, and fertilization. The present study aimed to determine whether pollen-grain distribution on the stigmas significantly affects the fruit shape.

Since the three stigmas and carpels of the watermelon are radially arranged, pollen distribution among the three stigmas was studied with relation to radial symmetry. Pear- or top-shaped fruits were not studied.

MATERIAL AND METHODS.—Watermelons (*Citrullus vulgaris*) of the Blue Ribbon variety were grown for this experiment at Davis, California, in 1942.

Pistillate flowers were covered with cloth bags the day before anthesis. Pollination was carried out between 8:00 and 10:00 A.M. the next morning, since pollen was difficult to obtain later in the day. The flowers were then labeled and bagged, as described by PORTER (3).

The flowers were pollinated in three ways: (*a*) pollen applied to all three stigmas; (*b*) one stigma cut off near its base and pollen applied to the other two; (*c*) two stigmas removed and pollen applied to the one remaining.

Pollen was spread abundantly over the stigmatic surfaces but was kept from the cuts where stigmas had been removed. In several melons, lanolin was spread on the cut surfaces to prevent pollen-grain germination. Since some injury resulted, however, the practice was not continued. Four melons so treated which matured (fig. 3, *C*, *K*; fig. 4, *B*, *P*) showed essentially the same seed distribution as if no lanolin had been used. The ovary was gently marked along one side with India ink to show excision of the stigmas, these marks later being renewed once.

Material for anatomical examination was fixed in Craf V (4). To trace pollen tubes, sections were stained with martius yellow-lacmoid blue (2). Other material was stained in iron haematoxylin.

Melons were harvested at or near maturity. The shape was recorded, especially near the blossom end, where deformity was most common. Shape and carpel size were also shown by a median cross-sectional tracing. The three carpels were then separated and their seeds counted. Empty seed coats were avoided, since they develop in parthenocarpic fruits and do not indicate fertilization (5).

The position of the carpels, which is not immediately obvious, must be ascertained for seed counts. In a cross section of a mature fruit, three conspicuous lines run from near the center almost to the edge, dividing the melon into three pie-shaped sectors, which, however, do not correspond to the three carpels. There is no obvious boundary between adjacent carpels. Figure 1*A* is the enlarged cross section of a fruit about 1.5 cm. in diameter. The broad ink marks indicate the limit of a single carpel. In figure 1*B* such a carpel has been cut free and teased apart to show the infolded edge of the car-

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pel wall and placental region bearing the ovules. Each stigma is directly above its carpel.

Because of a late crop, some melons had to be harvested before maturity.

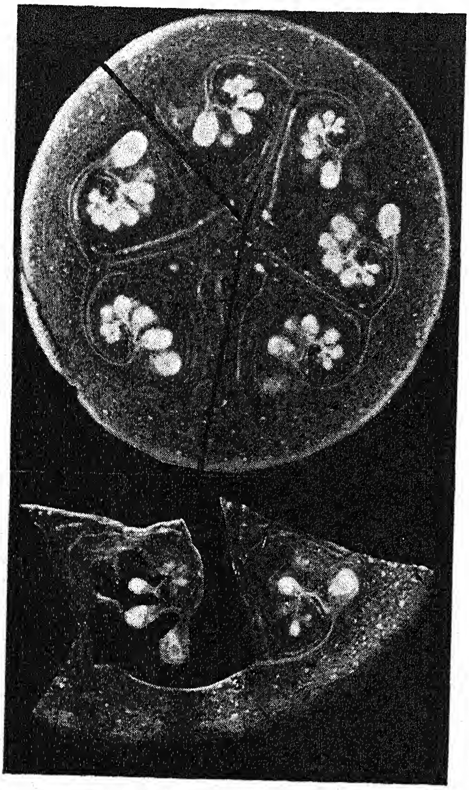

FIG. 1.—Top, cross section of young ovary, with limits of single carpel indicated by ink lines; bottom, single carpel cut free along lines indicated in top and teased apart to show infolded carpel, bearing the ovules.

Several were lost while small, being cracked and rotted because of extreme distortion. In figure 2*A* a single carpel (facing the observer) was unpollinated; in *B*, two carpels.

Investigation

GROWTH OF POLLEN TUBE.—Of primary importance was the direction of growth of the pollen tube. Does it necessarily fertilize an ovule located in the carpel on whose stigma the pollen grain was placed; and is the stimulative effect of pollen, pollen-tube growth, and fertilization confined mainly to the carpel where these processes occur?

The structure of the stigma, style, and ovary of the flower determines somewhat the path of the pollen tubes. The pistillate flowers have an inferior, usually tricarpellary, ovary, with a short thick style bearing three large stigmas.

FIG. 2.—Immature melons showing cracking caused by unequal rates of growth among carpels: left, single carpel facing the observer was unpollinated; right, two carpels.

KIRKWOOD (1), studying the cucurbitaceous genera *Melothera*, *Micrampelis*, and *Cyclanthera*, described a stylar canal; usually the pollen tubes move over the surface of the stylar lobes and enter the ovary through the canal, although when tubes are numerous they may penetrate the surrounding tissues. Once within the ovary, the tubes grow along the surface of the carpel walls and placentae to the ovules.

Watermelon has no stylar canal; the pollen tubes penetrate a solid trilobed mass of tissue extending through the upper portion of the ovary. Once within the ovary, the tubes travel over the carpel walls and placentae, which are covered with a rather distinct surface layer of starch-filled cells, as described for related

genera by KIRKWOOD (1). These surface layers become fused in *Citrullus vulgaris* after fertilization. Thus, anatomically there is no obvious reason why pollen tubes growing from a single stigma should not reach ovules in all three carpels.

In serial sections of ovaries where only one stigma had received pollen, tubes could be traced into all three lobes of the conducting tissue. They then passed into the ovarian cavity, whence they might reach any of the placental regions. Their distribution to those regions in the three carpels is, however, not random. Most pollen tubes grow directly downward from the stigmas where pollen grains were placed; thus a grain is far more likely to bring about the fertilization of an ovule of the carpel where it was deposited than an ovule of any other carpel.

SHAPE OF FRUITS AND DISTRIBUTION OF SEED.—As already indicated, flowers were pollinated in three ways. Figure 3 shows the control group of melons where all stigmas (four in several cases) were pollinated. A median cross-sectional slice of the melon is shown, the figures below each diagram indicating how many seeds were found in the carpels. In *A*, *E*, *F*, and *H* the last two figures indicating seed count are in one line separated by a comma. In *C* the seeds of only one carpel were counted. These melons varied greatly in shape. Most of the radial asymmetry occurred near the blossom end, one carpel being slightly flattened

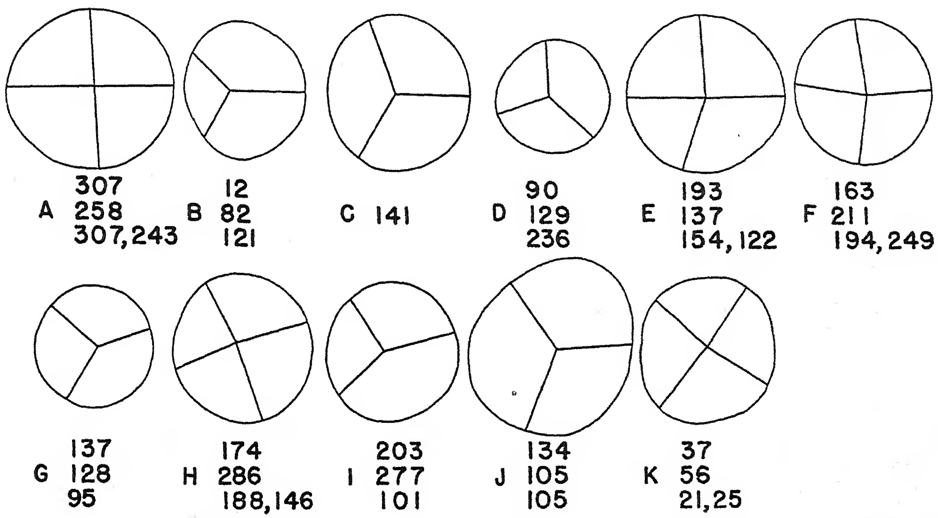

FIG. 3.—Median cross-sectional diagrams of control melons, showing area of carpels. Numbers below show seed count for each carpel. *A*, *E*, *F*, *H*, and *K* each have four carpels.

here and the blossom scar being located to one side of center. This was true of seven melons (*B*, *C*, *D*, *G*, *H*, *I*, *J*). The displacement of the blossom scar was measured as carefully as possible. For the seven it averaged 2.5 cm., with 3.0 cm. off center as maximum.

Judging from seed counts, the asymmetry may have resulted partly from differing amounts of fertilization in the carpels. In *B*, *D*, and *G* the smallest carpel (to the left in each diagram) contained the fewest seeds. In the other melons, the seed count does not refer to any specific carpel in the cross-sectional

diagram; and large differences in seed count are associated with differences in carpel size. Melons *A* and *K* are radially quite symmetrical, though *A* contains several times as many seeds as *K*. Melon *K*, however, was pear-shaped, all carpels being small toward the stem. Apparently shape is influenced more by relative number of developing seeds per carpel than by total number.

Figure 4 diagrams the melons in which one stigma was cut off and the remaining two were pollinated. The unpollinated carpel is always shown on the left. The numbers below indicate the number of seeds, the uppermost number referring to the left-hand carpel and the remaining numbers referring unspecifically to the other two.

All these melons except *F*, *H*, *M*, and *P* were asymmetrical to varying degrees. The blossom scar of the misshapen melons averaged 2.54 cm. off center, with 5 cm. as a maximum. Melon *N* was much curved, the blossom scar being displaced toward the stem end about 3 cm. from the apex. Except as just noted, most melons were misshapen, especially near the blossom end, where the unpollinated carpel was flat or sunken.

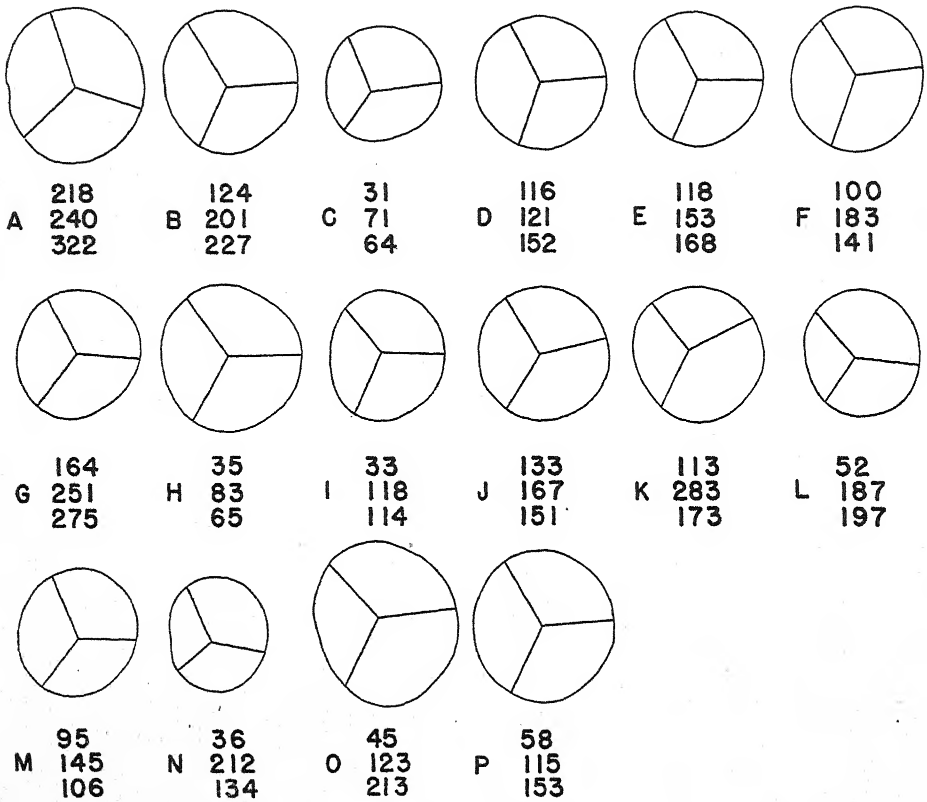

FIG. 4.—Median cross-sectional diagrams of melons pollinated on two stigmas only. Carpels at left were unpollinated. Upper number below each melon indicates seed count for unpollinated carpel; lower numbers refer unspecifically to the other two carpels.

Figure 5 shows melons developed from flowers that had two stigmas removed

(three removed in *I*) and the remaining stigma pollinated. In each diagram the pollinated carpel is at the left; and of the numbers below, the uppermost indicates the seed count in the left-hand carpel, while the remaining numbers refer unspecifically to the other two. Whereas melons *G* and *K* were symmetrical, the others curved or bulged on the side of the pollinated carpel; their blossom scars were pushed off center away from it, averaging 3.14 cm. off center, with a maximum of 5 cm. Melons *B* and *F* were so curved that the blossom scar was displaced 2.5 cm. and 5 cm., respectively, toward the stem end from the extreme apex. This group was more misshapen than the two groups previously discussed.

From the seed counts of pollinated and unpollinated carpels (figs. 4, 5) the percentage of pollen tubes that cross from a pollinated to an adjacent unpollinated carpel, bringing about fertilization, can be estimated. The percentage of pollen tubes so crossing, with its standard error for the melons pollinated on two stigmas (fig. 4), is 20.78 ± 1.69 per cent; for the melons pollinated on one stigma (fig. 5, melon *I* excluded) it is 21.87 ± 1.40 per cent.

For better evaluation of asymmetry, the carpel areas of the melons in figures 4 and 5 were measured with a polar planimeter. In the melons where two carpels were pollinated (fig. 4) the unpollinated carpels have a mean area (in arbitrary area units) of 69.41 units each; the pollinated, 83.51 units. In the melons where only one carpel was pollinated (fig. 5) the unpollinated carpels each have a mean area of 70.61 units; the pollinated, 122.27 units. Applying the *t*-test for unique samples, in both cases the difference in cross-sectional area between pollinated and unpollinated carpels is significant beyond the 1 per cent level.

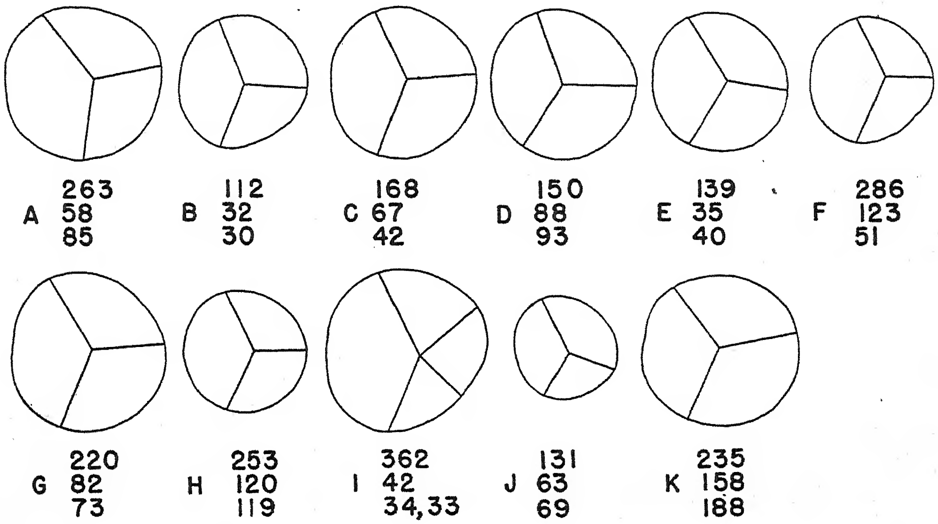

FIG. 5.—Median cross-sectional diagrams of melons pollinated on one stigma only. Pollinated carpel on left. Upper number below each melon indicates seed count for pollinated carpel; the other two numbers refer unspecifically to the remaining carpels.

Summary

1. The stigmas of the epigynous and usually tricarpellary flower of watermelon are distinct from one another. The conducting tissue of the style forms a solid three-lobed column, each lobe connecting a stigma with the carpel immediately beneath.

2. As shown by microscopic examination of this column, the pollen tubes growing downward from a specific stigma tend to be confined to the lobe of the conductive tissue, which is topped by the stigma where the pollen grains were placed. Certain pollen tubes turn laterally, however, both in the conductive tissue of the style and in the ovarian cavity, where they grow over the epidermal surface. Thus a pollen grain may bring about the fertilization of an ovule in a carpel other than the one on whose stigma it was placed.

3. According to seed counts from melons pollinated on only one or two stigmas, some 21–22 per cent of the pollen tubes will move laterally into each adjacent carpel.

4. The stimulus for enlarging a carpel is due, at least in part, to pollen tube growth and/or fertilization of ovules. This stimulus tends to be confined to the carpel where these processes take place. Although some pollen tubes cross from one carpel to another, large inequalities of pollen distribution among stigmas will cause differential growth among the three carpels, the fruit developing radial asymmetry, especially at the blossom end.

5. Analyzed by the *t*-test for unique samples, the mean difference in cross-sectional area between pollinated and unpollinated carpels of mature melons is significant beyond the 1 per cent level.

The writer is grateful to Dr. GLEN N. DAVIS for material and for helpful suggestions.

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LITERATURE CITED

1. KIRKWOOD, J. E., The pollen-tube in some of the Cucurbitaceae. Bull. Torr. Bot. Club 33:327–342. 1906.
2. NEBEL, B. R., Lacmoid-Mantius-yellow for staining pollen-tubes in the style. Stain Technol. 6:27–29. 1931.
3. PORTER, D. R., Watermelon breeding. Hilgardia 7:585–642. 1933.
4. SASS, J. E., Elements of botanical microtechnique. New York. 1940.
5. WONG, C. Y., Chemically induced parthenocarpy in certain horticultural plants, with special reference to watermelon. BOT. GAZ. 103:64–86. 1941.

MEGAGAMETOPHYTE OF CLINTONIA[1]

FRANK H. SMITH

Development of the megagametophyte of *Clintonia borealis* has been described by R. W. SMITH (3). According to his description, two unlike daughter nuclei are produced in the first reduction division. The nucleus formed at the chalazal end of the spindle consists of a dense chromatic mass, while that at the micropylar end develops into a typical interphase nucleus. As a result of the second division the chalazal nucleus produces two groups of daughter chromosomes which remain compacted into dense chromatic masses. The micropylar nucleus divides at the same time to form both a normal nucleus at the micropylar pole of the spindle and another mass of chromatic material at the opposite pole. Thus, of the four megaspore nuclei formed, the three inner ones are degenerate. No cell walls are formed following either division. The functional nucleus divides twice to form the nuclei of the egg apparatus and a single polar nucleus. One or more bodies representing the degenerate megaspore nuclei persist in the cytoplasm of the primary endosperm cell.

The reliability of these observations and interpretations has been questioned by many investigators, particularly by those interested in determining a system of classification of the different types of megagametophytes. SCHNARF (2) states: "A reinvestigation is needed, since the alleged course of development does not agree with that of closely related plants." MAHESHWARI (1) attempted to interpret the figures published by SMITH in terms of the *Fritillaria*-type megagametophyte. Much of the doubt cast upon SMITH's observations is probably due to the fact that he illustrated principally only telophase stages of most of the nuclear divisions involved.

In view of the confusion in the literature regarding the megagametophyte of this genus, and the scarcity of critical stages observed by SMITH, material of *Clintonia uniflora* (Schult.) Kunth. was collected for study. The ovaries were fixed in Navashin's solution, dehydrated, and imbedded by the tertiary butyl-alcohol technique. Sections were cut 10-30 μ and stained with safranin and fast green.

Observations

The haploid number of chromosomes in *C. uniflora* is 14 (figs. 1, 11). The archesporial cell is hypodermal and functions directly as the megaspore mother cell (fig. 2). The chromosome pairs are arranged regularly at the equatorial plate of the first reduction division (fig. 3). This division proceeds in a normal manner up to the point where the chromosomes are compactly grouped at the poles of the spindle (fig. 4). Here, however, the two groups behave differently. The chromosomes at the pole of the spindle nearest the micropylar end of the mother cell undergo the usual telophase changes and become dispersed to form a typical interphase nucleus (fig. 5), while those at the chalazal pole remain compactly grouped because of suppression of the telophase. The midportion of the spindle persists in the cytoplasm, but no indications of a cell plate, as described by SMITH, were observed.

It seems likely that the telophase of the first division is merged with the prophase of the second and that both stages are rapidly completed. No prophase stages of the second division were de-

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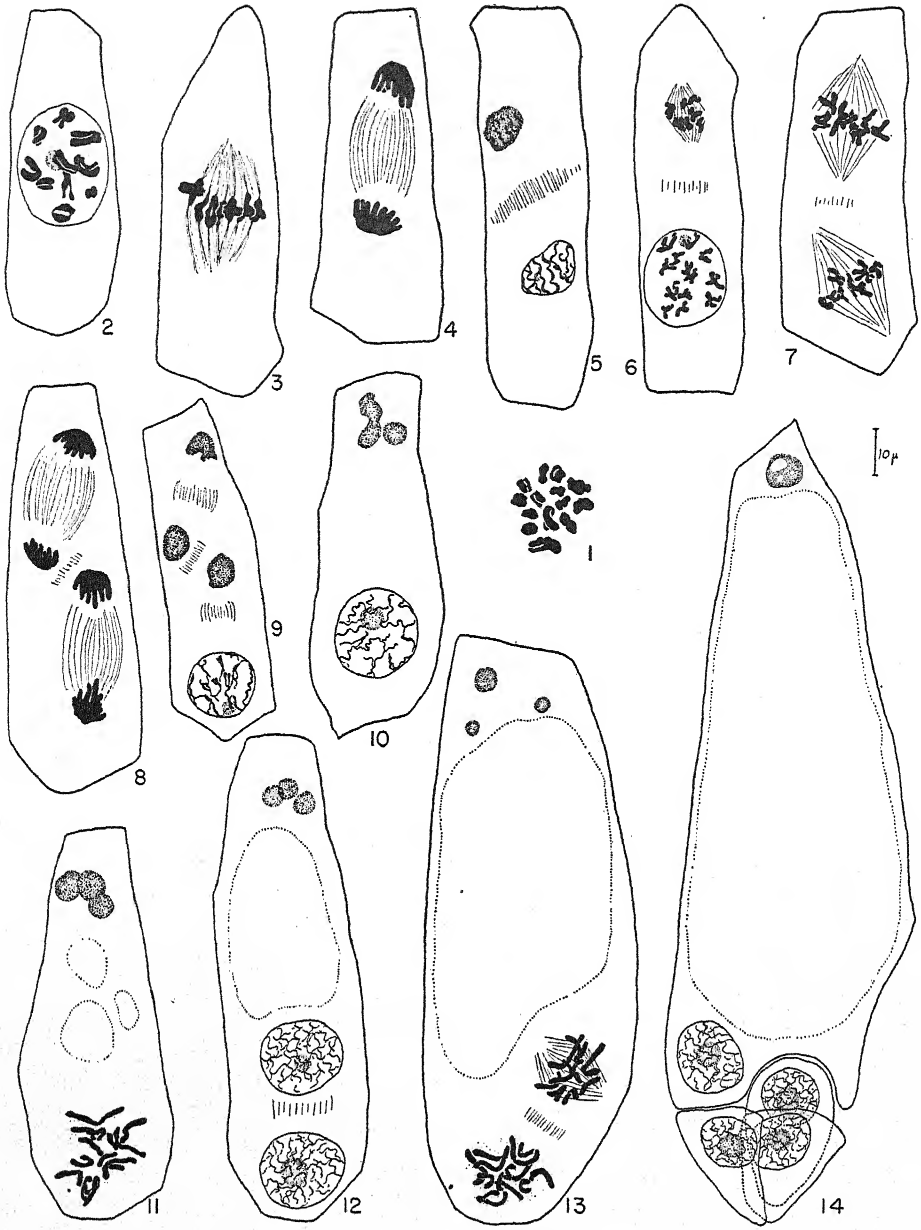

FIGS. 1–14.—Fig. 1, meiotic metaphase I in microspore mother cell. Figs. 2–14, development of megagametophyte: fig. 2, diakinesis; fig. 3, metaphase I; fig. 4, late anaphase I; fig. 5, telophase I; fig. 6, prophase II; fig. 7, metaphase II; fig. 8, late anaphase II; fig. 9, late telophase II; fig. 10, megaspore containing one functional and three degenerate megaspore nuclei; fig. 11, metaphase of division of megaspore nucleus; fig. 12, late telophase of division of megaspore nucleus; fig. 13, metaphase of second division of megaspore nucleus; fig. 14, nearly mature megagametophyte.

scribed by SMITH, and very few were found in the present study. The compactly arranged chromosomes at the chalazal end of the cell separate from one another while the nucleus at the micropylar end passes rapidly through the usual prophase stages (fig. 6). The upper spindle develops before the lower one, but the metaphase is reached at approximately the same time in both (fig. 7). Thus, in spite of the differences in behavior of the micropylar and chalazal groups of chromosomes of the first reduction division, the spindles of the second division are both normal. They are usually arranged along approximately the same axis. So far as could be determined, each spindle contains the haploid complement of 14 chromosomes. The chromosomes then separate regularly into daughter halves, which move to opposite poles of each spindle (fig. 8). As in the first division, the telophase is suppressed in some of the chromosome groups. The chromosomes at the micropylar end of the outermost spindle undergo the normal telophase changes to form a typical metabolic nucleus (figs. 9, 10). The chromosomes of each of the three remaining groups become compactly arranged and appear gradually to fuse together and lose their identity in a mass of heavily staining material (fig. 9). Persistent fibers of both the first and second divisions are evident at this time but soon disappear without forming any cell plates.

The megaspores resulting from the reduction divisions are thus represented by one normal nucleus at the micropylar end of the cell and three masses of chromatic material at the center or the chalazal end. In *C. borealis*, SMITH noted that in every cell observed at this stage the three chromatic masses were present but that in later stages usually less than three were found. In *C. uniflora* frequent examples of the fusion of these bodies were observed (figs. 10, 11), although fusion does not always occur. Thus during later stages of the development of the megagametophyte there may be one, two, or three chromatic masses resulting from the fusion of all, some, or none of the degenerate megaspore nuclei. The appearance of one large and two smaller bodies in the same cell (fig. 13; and figs. 8, 9 of SMITH) is probably due to the fact that the products of the second reduction division in the chalazal end of the cell tend to become vacuolated and absorbed earlier than the other degenerate nucleus. During later stages any chromatic masses which are present become vacuolated and undoubtedly are eventually absorbed. They are still present, however, at the time of fertilization.

Division of the functional megaspore nucleus proceeds in the usual manner (fig. 11), and persistent spindle fibers of this division are evident between the daughter nuclei (fig. 12). These nuclei then divide (fig. 13), and cell plates are produced by the fibers of all spindles to cut out the egg apparatus and the primary endosperm cell. Thus the megagametophyte is composed of four cells (fig. 14), with the primary endosperm cell containing the polar nucleus and one, two, or three chromatic bodies representing the degenerate megaspore nuclei.

Discussion

Much of the confusion regarding the nature of the megagametophyte of *Clintonia* may possibly be traced to its unfortunate comparison by SMITH with the megagametophyte of *Oenothera*. SCHNARF (2) apparently misinterpreted this comparison and stated that "according to SMITH .... *Clintonia borealis* follows the *Oenothera*-type." SMITH emphasized

that, while the normal nucleus and the three degenerate nuclei produced by the reduction divisions represent four megaspores, there are no walls between them. The principal point of comparison of the two types seems to be that it is the micropylar megaspore nucleus in each case that functions to produce the quartet. The same, however, may be said of the *Gunnera-*, *Pyrethrum-*, *Majanthemum-*, and *Fritillaria*-type megagametophytes in SCHNARF's system of classification. The presence of the degenerate megaspore nuclei in the antipodal end of the megagametophyte indicates a closer relationship of *Clintonia* to these rather than to *Oenothera*.

MAHESHWARI (1) states, regarding SMITH's observations on *C. borealis*, "This satisfies the requirements of the *Oenothera*-type, and if SMITH's observations are correct *Clintonia* should be regarded as an interesting example of a monosporic tetranucleate embryo sac." The present work on *C. uniflora* shows that SMITH's observations are probably correct. He stated definitely, regarding the products of the reduction divisions, "No one doubts that these are megaspores, simply because three of them or their products disintegrate." This statement and his subsequent discussion indicate that he considered the megagametophyte to be tetrasporic, with three of the spores represented only by degenerate nuclei.

However, MAHESHWARI's interpretation of SMITH's illustrations in terms of the *Fritillaria*-type megagametophyte is not entirely incorrect. According to this interpretation the development follows the *Fritillaria*-type up to the completion of the secondary 4-nucleate stage. The two micropylar nuclei then divide to form the quartet, while the two chalazal nuclei fail to divide. Thus *Clintonia*, on the basis of this interpretation, would have a reduced megagametophyte of the *Fritillaria*-type with six nuclei instead of the normal eight. The present work has shown that a secondary 4-nucleate stage, in terms of the *Fritillaria*-type, does not exist in *Clintonia*.

It would appear from the observations of SMITH on *C. borealis*, and from the present investigation of *C. uniflora*, that *Clintonia* does have a megagametophyte reduced from the *Fritillaria*-type. However, the reduction is much greater than that postulated by MAHESHWARI. The latter discusses a reduction series in this type of megagametophyte that leads logically to the situation in *Clintonia*. He states that in *Gagea* the lowest nucleus of the secondary 4-nucleate stage does not divide, so that a 7-nucleate megagametophyte is formed, while in *Gagea ova* the chalazal pair of nuclei of the secondary 4-nucleate stage sometimes undergo no further division and the megagametophyte is 6-nucleate. In a footnote he adds that in *Caulophyllum robustum*, as described by MAURITZON, the three chalazal nuclei of the primary 4-nucleate stage fuse to form a triploid nucleus which does not undergo further division. The megagametophyte is thus 5-nucleate.

Clintonia shows the same situation as that described for *Caulophyllum*, except that in *Clintonia* the three chalazal megaspore nuclei are degenerate and do not always fuse.

Summary

1. Observations by R. W. SMITH on the megagametophyte of *Clintonia borealis*, which have been much doubted, are probably essentially correct, since *C. uniflora* follows the same pattern of development as he described for *C. borealis*.

2. *Clintonia* has a tetrasporic megagametophyte which probably represents a much reduced form of the *Fritillaria*-type. The nucleus of the megaspore mother cell divides to form four megaspore nuclei, of which the three toward the chalazal end are represented only by densely chromatic masses. All or some of these masses may fuse and then persist as one or more bodies in the cytoplasm of the primary endosperm cell. The micropylar megaspore nucleus divides twice, to produce the quartet of two synergids, the egg, and a single polar nucleus.

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LITERATURE CITED

1. Maheshwari, P., A critical review of the types of embryo sacs in angiosperms. New Phytol. **36**: 359–417. 1937.
2. Schnarf, K., Contemporary understanding of embryo-sac development among angiosperms. Bot. Rev. **2**: 565–585. 1936.
3. Smith, R. W., The tetranucleate embryo sac of *Clintonia*. Bot. Gaz. **52**: 209–217. 1911.

HISTOLOGICAL AND CYTOLOGICAL RESPONSES OF ROOTS TO GROWTH-REGULATING SUBSTANCES

CONTRIBUTIONS FROM THE HULL BOTANICAL LABORATORY 557

WILLIAM M. CARLTON

Introduction

Gross responses of plant organs to growth-regulating substances have been studied widely during the past decade. Histological and cytological studies of the effects of such substances on stems and other aerial organs have been reported by many investigators (1, 2, 4, 9, 11, 12, 13, 14, 15). Studies of the effects of such substances on plant roots, however, have been limited largely to gross observations on the stimulation or inhibition of root elongation, the development of laterals, and the physiological activity of the roots. Only recently have the histological and cytological changes induced been considered.

According to THIMANN (20), NIELSON first demonstrated (in 1930) the inhibition of root elongation by the external application of auxins, using in his treatment a crude extract from *Rhizopus* cultures. Later (in 1934) KÖGL, HAAGEN-SMIT, and ERXLEBEN found that pure auxin a, auxin b, and indole(3)acetic acid had a similar effect. THIMANN found that indene(3)acetic acid applied externally also inhibited elongation, and he advanced the suggestion that perhaps this inhibition is a general property of growth-regulating substances.

ZIMMERMAN and HITCHCOCK (21) found that the normally unbranching aerial roots of grape could be induced to form numerous lateral roots and that elongation was retarded by treatment of the region with any of several chemicals in lanolin paste or by immersing the root tips in aqueous solutions of the substances.

LANE (16) found that indole(3)acetic acid is a specific inhibitor of root growth in young *Avena* seedlings and that the inhibition is accompanied by some thickening of the root. Stunting effects on roots also were reported by THIMANN, MARMER (19), MACHT and GRUMBEIN (18), and BONNER and KOEPFLI (5). These effects were studied chiefly on roots of cereal grains and legumes. BORGSTROM (6) investigated the effects of a number of growth substances on bulb-forming plants (*Allium cepa*, *A. fistulosum*, and *Crocus*), determining effective concentrations, toxicity limits, and gross morphological effects. He found that elongation of roots was inhibited by treatment with all but the most dilute concentrations, and inhibited elongation often was accompanied by enlargement of the root tips.

The effectiveness of growth-regulating substances in the induction of lateral root formation has been observed in various plants (21, 20, 16, 19, 8, 6). MARMER (19) reported excessive root-hair formation following treatment of wheat seedlings with indoleacetic, indolebutyric, and indolepropionic acids at pH 4.6 but not at pH 7.5.

The histological and cytological responses of roots of species of *Allium* to several chemicals has been investigated by LEVAN (17). BURSTRÖM (7) has discussed the morphological changes induced in roots of wheat following treatment with heteroauxin.

The present investigation was undertaken for the purpose of extending information on the histological and cytological changes which occur in roots following treatment with various growth-regulating substances.

Material and methods

The roots used were those of bulb-forming monocotyledons. The species selected were *Allium cepa*, *Narcissus* (var. Paper White), and *Tulipa* (vars. John Ruskin and Louis XIV), but not all were treated with all of the chemicals used.

Six growth-regulating substances were used: α-naphthaleneacetic acid, indole(3)acetic acid, indole(3)butyric acid, β-naphthoxyacetic acid, α-naphthyl acetamide, and tryptophane. They were used in concentrations of 0.001 or 0.002%, 10 or 20 p.p.m., dissolved in a three-salt nutrient solution. Because of the difficulty in getting some of the substances into solution in an aqueous medium, all but tryptophane and naphthyl acetamide were first dissolved in small amounts of ethyl alcohol, in a manner similar to that employed by MACHT and GRUMBEIN (18); later, measured amounts of the alcoholic solutions were pipetted (with constant agitation) into definite volumes of nutrient solution to give the desired concentrations. The naphthyl acetamide, being relatively insoluble in ethyl alcohol, was put into solution in hot n-butyl alcohol. Tryptophane was dissolved directly in the measured volume of nutrient solution.

The bulbs were rooted by suspending them on wire-gauze racks over containers of nutrient solution so that the basal portions were submerged. When the roots had reached a satisfactory length, 1 inch or more in case of *Allium* and *Narcissus* and somewhat less in the case of *Tulipa*, individual bulbs were removed from the racks, rinsed with water, and suspended in tumblers so that the roots were completely immersed in nutrient solution to which the respective substances had been added.

Length of treatment varied from 24 to 72 hours, being generally 48 hours. At the conclusion of the treatment the bulbs were removed from the growth-substance solution, rinsed in distilled water, and transferred to tumblers of regular nutrient solution. Roots were cut at even intervals from 24 to 216 hours after the bulbs were replaced in nutrient solution, and fixed in Navashin's solution under reduced pressure.

The fixed roots were dehydrated by the tertiary butyl-alcohol method, imbedded in paraffin, sectioned, and stained in a modification of Flemming's triple stain. All sections were cut at 15 μ, with the exception of the longitudinal sections of the *Tulipa* roots. These, because of their small size, were sectioned at 10 μ.

Observations

GROSS RESPONSES

No definite responses of the roots were noticed for approximately 24 hours after replacing the bulbs in nutrient solution following treatment with the growth substance solutions; but between 24 and 72 hours after their return, several changes were noted.

All the chemicals in the concentrations used resulted in inhibition of root elongation, either temporary or permanent. Following treatment with indoleacetic acid the roots soon began to elongate, and in some cases the root tips grew as much as 2 inches during the subsequent 3 weeks. The roots treated with the other chemicals showed no later elongation.

Often a swelling was observed near the root tip; the time of occurrence and the amount varied in the different species. *Allium* showed the quickest response, but *Narcissus* reacted almost as rapidly. The amount of terminal enlargement in

the roots of *Tulipa* was so slight as to be hardly discernible, and histological examination of longitudinal sections were required to determine it.

The terminal swellings began about 24 hours after replacement of the bulbs in nutrient solution. Their size increased and reached a maximum in 1–3 days.

The enlargements which developed on roots of *Allium* and *Narcissus* as a result of treatment with the indoleacetic, indolebutyric, naphthaleneacetic, and naphthoxyacetic acids were similar in appearance and somewhat larger in diameter than were those induced by tryptophane. They were frequently twice the diameter of the root and sometimes scarcely longer than thick, while those induced by tryptophane were slender and often $\frac{1}{2}$ inch or more in length.

About 200 hours after treatment, the enlargements at the tips of *Narcissus* roots treated with naphthaleneacetic began to produce root hairs. At 264 hours the enlargements were almost completely covered with them, none more than a small fraction of a millimeter in length. Root hairs were not induced by the other chemicals, nor were they formed on roots of the other species.

Root primordia and secondary roots developed only on *Allium*. This genus normally forms secondary roots, but there were more primordia, and these were closer to the terminal meristem than in untreated bulbs. The secondary roots were much stunted and enlarged, and some of them had primordia (fig. 4).

Histological and cytological responses

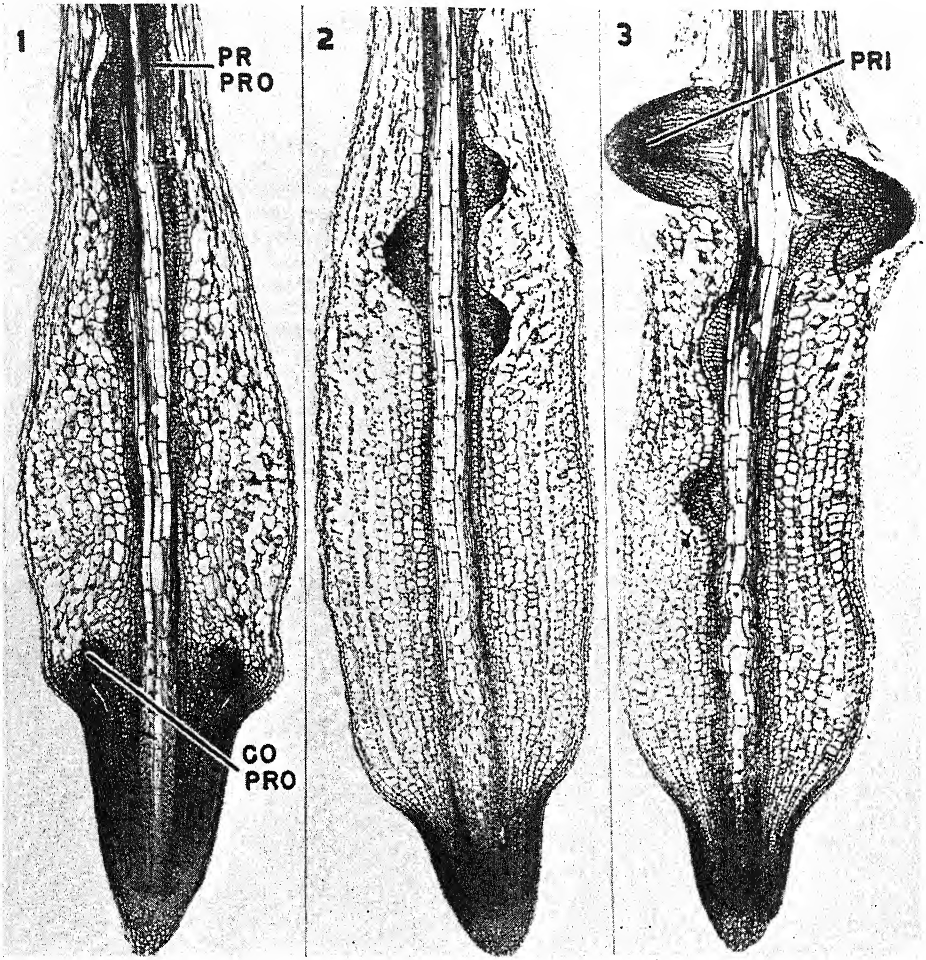
In *Allium* the characteristic enlargement of the root tip following treatment reaches maximum size within 48 hours. Transition from normal tip to swollen area is rather gradual and covers about 20 cell lengths. Within 48 hours the pericyclic cells for some distance back from the apex of the root undergo transverse divisions which result in the formation of longitudinal rows of short, meristematic cells, which divide in several planes (figs. 1, 2). Tangential division of the cells of the pericycle becomes prominent in 72 hours, and within 96 hours definite root primordia are developed. About 264 hours are required for the primordia to grow through to the epidermis (fig. 8). Within 360 hours many have begun to emerge (figs. 3, 5, 7). Some are much enlarged and in turn have formed primordia (fig. 4).

While cell proliferation is much more frequent in the pericycle than in any other tissue of the root, it may also occur in other tissues, such as the cortex (figs. 1, 6). Proliferation, when it occurred, was in the form of a band centrifugal to the stele and extending almost to the epidermis (figs. 1, 6). Such divisions in the cortex of the embryonic tip occur early in ontogeny. They are at first radial or diagonal, but in older material many divide tangentially. Division is not pronounced in the larger cells of the swollen region. Some division also occurs in the mature cells far above the terminal enlargement.

Roots of John Ruskin and Louis XIV *Tulipa* do not develop noticeable swollen tips after treatment. The earliest response is the altered cell polarity in the immature cortex. As early as 48 hours after treatment, and in all cases thereafter, these cells show evidence of recently completed divisions in all planes. Close to the tip the divisions are predominantly radial or diagonal, but at higher levels many of them are tangential (figs. 9, 10). For the first 48 hours after treatment the divisions are limited almost exclusively to the meristematic re-

gion, but after 72 hours divisions in several planes occur in mature cells (fig. 10).

formed remaining slender and undergoing no enlargement. Subsequently the pericyclic cells divide further and form



FIGS. 1–3.—Longisections of *Allium* root tips treated with 0.001% naphthaleneacetic acid: Fig. 1, 120 hours after treatment, showing proliferation of pericycle and cortex. Fig. 2, 216 hours, showing root primordia. Fig. 3, 360 hours, showing primordia emerging. Figs. 2 and 3 show transition from normal to enlarged regions. *Pr pro*, proliferation of pericycle; *Co pro*, proliferation of cortex; *Pri*, root primordium.

Transverse divisions are initiated in the pericycle approximately 72 hours after treatment and continue for 72 hours or more. These divisions are confined to limited areas. Tangential divisions first occur about 168 hours after treatment, the daughter cells thus ridges of densely packed cells which often extend 200 μ or more along the stele. Whether root primordia are initiated is uncertain. No definite organization was seen in the ridges of meristematic cells, but it is possible that root primordia might eventually differentiate. Iso-

lated cells of the endodermis undergo tangential division within 96 hours after treatment, and within 144 hours many have divided.

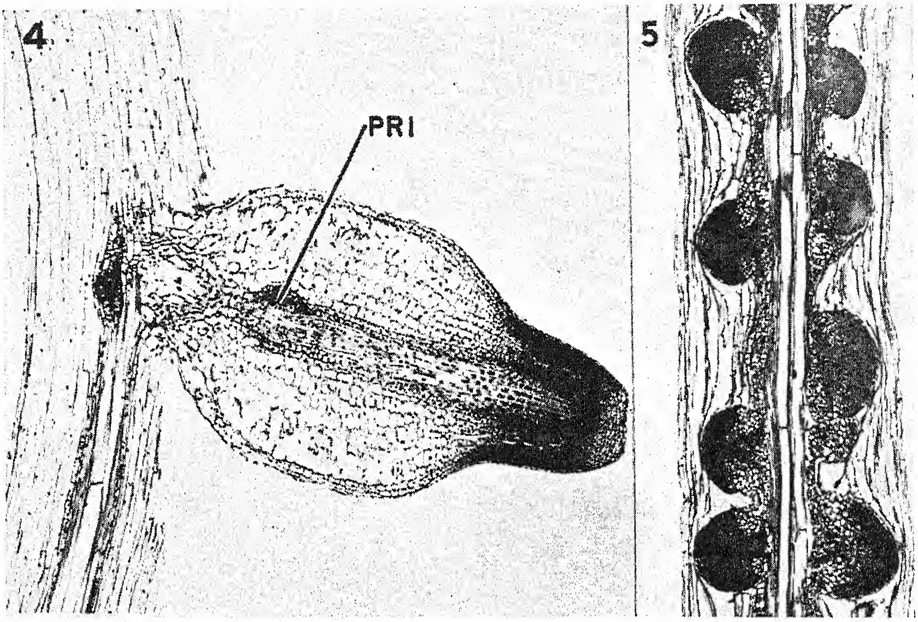
In *Narcissus* roots no terminal swelling appears until 72 hours after treatment, and the maximum size is not reached for approximately 144 hours. Few or numerous root hairs develop on the tumor after 200 hours. Their maximum length never exceeds a small fraction of a millimeter.

Some division occurs in mature cells of the cortex, particularly in the early stages. No response was observed in either the pericycle or the endodermis.

INDOLE(3)ACETIC ACID.—Treatment with 0.001% for 24 hours was applied to John Ruskin *Tulipa* and *Narcissus* in the manner previously described. Roots were collected and fixed at the conclusion of the treatment, and at regular intervals up to 192 hours after the bulbs were replaced in nutrient solution.

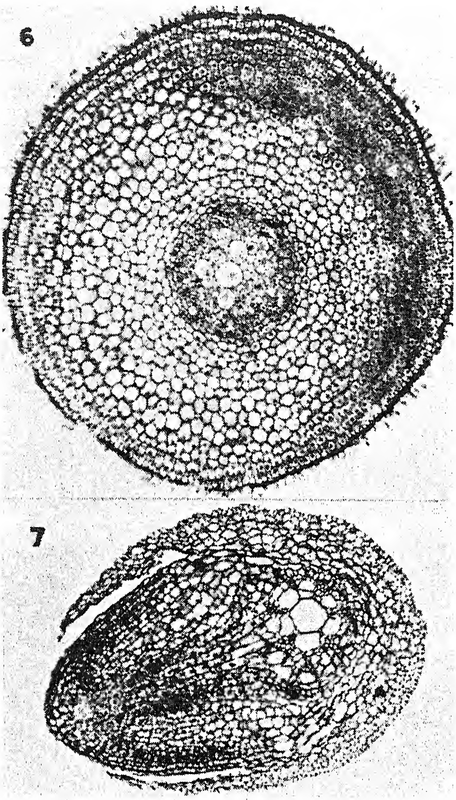
Treatment does not result in tumor formation on roots of the John Ruskin variety. In fact, little variation from normal can be detected. Divisions in the cortex of the meristematic region appear about 12 hours after treatment and eventually increase in number. These divisions at first are mostly radial, but later numerous tangential and diagonal ones occur. The daughter cells do not enlarge, and division in any cortical cell is completely independent of division in the adjacent cells.

Radial divisions in both pericycle and endodermis begin as early as 12 hours after treatment. Cells of the endodermis show tangential divisions 48 hours after treatment, and about 24 hours later



FIGS. 4, 5.—Longisections of mature regions of *Allium* roots 360 hours after treatment with naphthalene-acetic acid. Fig. 4, induced lateral root with young primordium developing. Fig. 5, section about 3 cm. above root apex with numerous root primordia.

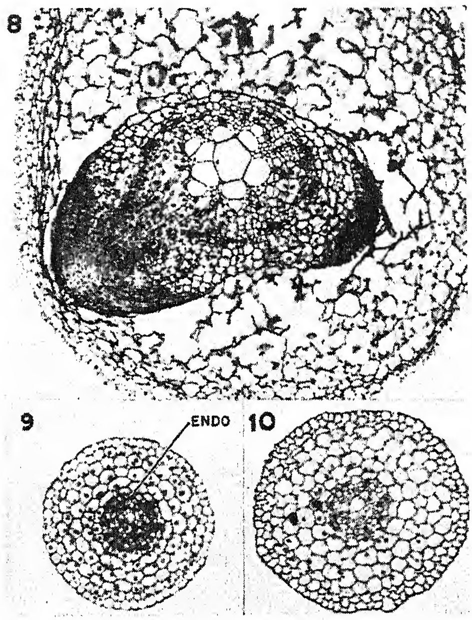
those of the pericycle also. At the same time, or perhaps somewhat earlier, the pericyclic cells undergo transverse division, resulting in rows of cubical cells



Figs. 6, 7.—Transection of *Allium* root treated with naphthaleneacetic acid: Fig. 6, 120 hours after treatment, cut through base of tumor to show proliferation of cortex (cf. fig. 1) and early divisions in pericycle and endodermis. Fig. 7, 360 hours after treatment, showing emerging root primordium and development of vascular connections.

which are densely filled with cytoplasm and apparently meristematic. There are continued radial and tangential divisions in these tissues 120–192 hours after treatment, and the cortical cells adjacent to the endodermis also are affected. Proliferation is general, resulting in a compact band of four to six rows of cells around the stele. In more mature portions general proliferation does not occur, but localized regions of meristematic cells may indicate differentiation of root primordia.

Roots of *Narcissus* show no terminal enlargement at the conclusion of the

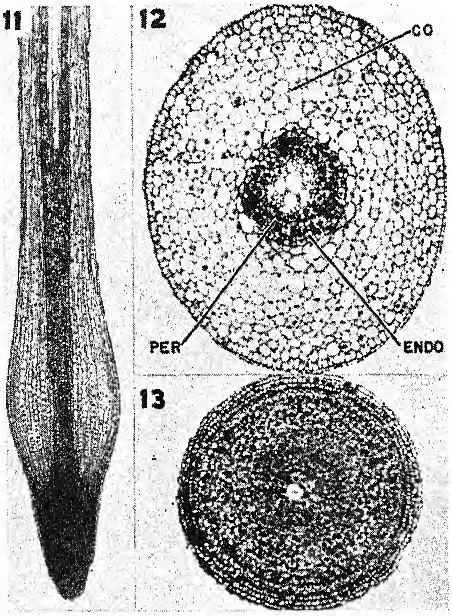


Figs. 8–10.—Transection of roots treated with naphthaleneacetic acid: Fig. 8, *Allium*, 264 hours after treatment, showing developing primordia and torn cortex. Fig. 9, *Tulipa* Louis XIV, 120 hours, showing newly divided cells in endodermis and cortex and "apparent" binucleate condition in some cortical cells. Fig. 10, Louis XIV, 144 hours, showing effects of division in cortex and mitosis in mature cell. *Endo*, endodermis.

treatment, but swelling is initiated within 24 hours and reaches its maximum in about 48 hours. Tumor formation results from enlargement of the cells of the cortex, with no indication of increase in the number of rows of cells in a longitudinal section. The treatment exerts a temporary stunting effect, stopping elongation for approximately 96 hours; but this is of short duration and then elongation is resumed. With renewal of elongation, the

tumor is left farther and farther behind the growing tip. Apparently, however, no further change occurs in its cells, for they remain almost isodiametric.

A few tangential and radial divisions occur in the cortical cells. The peripheral four or five rows undergo less enlargement than those nearer the center, and as a result the swollen region is bounded by a band of compact cells which remain active longer than most of the others.



FIGS. 11–13.—*Allium* root: Fig. 11, 72 hours after treatment with indolebutyric acid, showing proliferation of pericycle and transition from normal tip to tumor. Fig. 12, transection through tumor, 72 hours after treatment with indolebutyric. Fig. 13, transection just below tumor, 168 hours after treatment, showing divisions of cortical cells in all planes. *Co*, cortex; *Endo*, endodermis; *Per*, pericycle.

Intercellular spaces, which occur even in the meristematic region of the root tip, are much larger in the swollen portion of the root. Mature cells are stimulated to divide in many instances, such divisions occurring as far as 16 mm. above the root tip, a considerably greater distance than is usual. In all instances division in mature cells occurs transversely.

INDOLE(3)BUTYRIC ACID.—Rooted bulbs of *Allium*, Louis XIV *Tulipa*, and *Narcissus* were treated with a 0.001% solution of this acid for 24 hours. Following treatment, roots were collected and fixed at 48-hour intervals up to and including 216 hours.

Swelling induced in *Allium* roots by this treatment reaches maximum size in approximately 24 hours, forming a spindle-shaped enlargement just above the root tip (fig. 11). First the pericyclic cells undergo repeated transverse divisions to form blocks of short meristematic cells. Tangential divisions follow shortly, forming a compact band several cells in thickness around the stele (fig. 12). This is accompanied by tangential divisions in the endodermis and, to some extent, by similar divisions of the inner cells of the cortex. The band thus formed varies in thickness from two to twelve cells and has an irregular external boundary. Development of this compact band of tissue is generally limited to the elongation and maturation regions. In the mature regions, within 72–216 hours after treatment, distinct areas of proliferation are formed. At first these are no more than compact groups of actively dividing cells, but later there are definite organization of tissues and differentiation of root primordia.

Mature cells of the cortex divide, but all divisions appear to be oriented normally in a transverse plane. Divisions in all planes occur in the immature cortical cells, the number progressively increasing. At 168 hours almost all the cells in the cortex of the meristematic region have undergone one or more divisions (fig. 13).

The only responses of roots of Louis

XIV *Tulipa* to treatment consist of a few radial and tangential divisions in the young cortical cells and a few transverse divisions in the mature region.

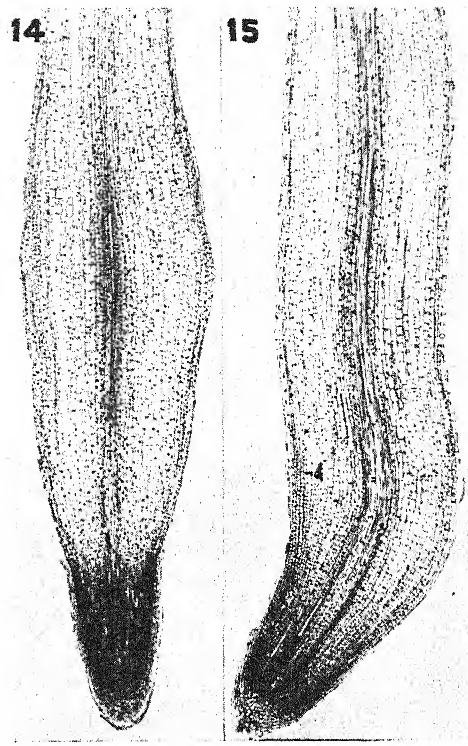
Roots of *Narcissus* show no pronounced swellings 24 hours after treatment, but these are obvious within 72 hours. They are formed by the enlargement of individual cells rather than by increase in their number. There is a temporary stunting of root growth which lasts for approximately 168 hours, after which there is some slight renewal of elongation. Both radial and tangential divisions occur in the cells of the immature cortex but never abundantly. There are also a few transverse divisions in the mature cortical cells.

Transverse sections through the tumor show that the five or six peripheral rows of cortical cells are much more compactly arranged than are those nearer the stele. Intercellular spaces become numerous and large. Development of a limited number of stubby root hairs occurs on the lower portion of the tumor 120–216 hours after treatment.

β-NAPHTHOXYACETIC ACID.—Bulbs of both varieties of *Tulipa* and *Narcissus*, with abundant root development, were treated for 48 hours with a 0.002% solution of this acid. Roots were collected and fixed at 24-hour intervals from 48 to 144 hours.

Narcissus roots usually respond to treatment by tumor formation (figs. 14, 15), but there are exceptions where no swelling occurs. The roots are apparently permanently stunted. The tumors are formed by the enlargement of cortical cells, chiefly in a radial plane. Transition from the normal root tip may occur gradually or abruptly, involving only a few cell lengths. Roots collected and fixed at 72 and 120 hours after treatment have sparse development of root hairs on the lower part of the tumor, and their maximum length is about 0.1 mm.

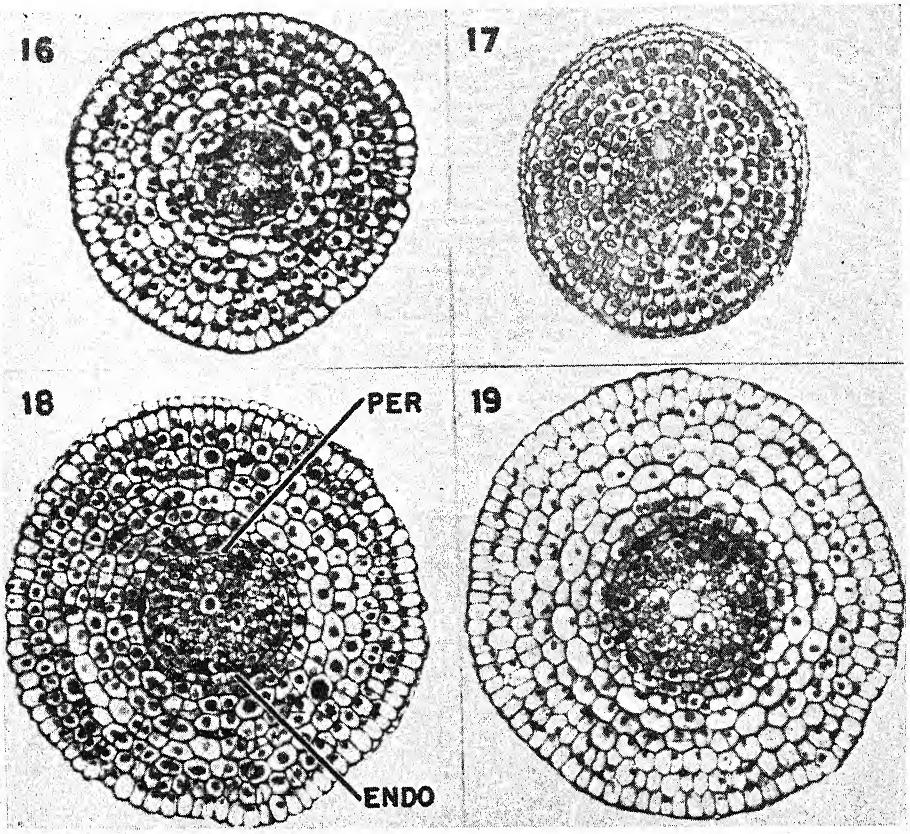
Transverse sections indicate that cell development and mitosis are normal during early phases (up to 96 hours), but in material fixed 120 hours or more after treatment there are numerous divisions in all planes in the meristematic region. Mature cortical cells as far as 10 mm. from the root tip are stimulated to divide. Intercellular spaces occur in the swollen portion of the root. No changes were induced in the cells of the pericycle or in the stele of any root given this treatment.



FIGS. 14, 15.—*Narcissus* root: Fig. 14, longitudinal section, 120 hours after treatment with naphthoxyacetic acid. Fig. 15, 144 hours after treatment with naphthyl acetamide.

Pronounced terminal swelling does not occur in roots of Louis XIV. Cortical

cells just back of the root tip undergo numerous divisions, but the daughter cells show little enlargement. The earliest divisions appear to be radial and occur mainly toward the periphery (figs. 16, 17), but of those occurring later, many are tangential (figs. 18, 19). Pericyclic cells are stimulated to divide transversely in certain regions, forming short rows of nearly isodiametric cells with large nuclei and dense cytoplasm. In older roots, killed 120 hours or more after treatment, both pericyclic and endodermal cells divide both radially and tangentially; the daughter cells thus formed remain densely filled with cytoplasm. These regions often appear to be associated with the protoxylem points, which might indicate early stages in the formation of root primordia; but development is so slight that no definite conclusions can be drawn.



FIGS. 16–19.—Transections of *Tulipa* Louis XIV roots treated with naphthoxyacetic acid: Fig. 16, 48 hours after treatment, showing numerous recently completed divisions in cortex. Fig. 17, 96 hours; similar divisions. Fig. 18, 120 hours; divisions in all directions in cortex and initiation of divisions in endodermis. Fig. 19, 144 hours; section cut at higher level, showing divisions in endodermis and pericycle. *Endo*, endodermis; *Per*, pericycle.

Mature cells of the cortex are stimulated to divide as far as 10 mm. from the root tip and in a number of planes.

Roots of John Ruskin *Tulipa* respond

similarly but somewhat more slowly than those of Louis XIV. Pericyclic cells divide frequently, and the daughter cells are filled with dense cytoplasm; but despite their meristematic appearance, no proliferation appears to take place.

α-NAPHTHYL ACETAMIDE.—Rooted bulbs of *Allium*, Louis XIV *Tulipa*, and *Narcissus* were treated for 48 hours with a 0.001% solution. Roots were collected and killed at 48-hour intervals up to 192 hours.

Allium roots show considerable swelling, forming bulbous tumors. Lateral root primordia develop rather quickly, and mitosis occurs in cells throughout the terminal 10 mm. of the roots, both in the stele and in the cortex. The tumors formed on the roots are similar to those previously described and result from cell enlargement rather than from increase in cell number. Transition from the normal root tip takes place within 10–12 cell lengths. Few of the enlarged cells of the cortex show either recently completed divisions or mitotic figures, indicating little meristematic activity.

Root primordia are initiated within 48 hours following treatment, but the number at this time is few; at 144 hours after treatment their number has increased considerably. The position of the primordia appears to have no effect on their size or rate of development, since those nearest the root tip are not necessarily the youngest or the smallest. Within 192 hours after treatment, some primordia have pushed halfway through the cortex. The steps in the development of primordia are essentially the same as those previously discussed, but none developed sufficiently to emerge from the main root.

Some enlargement of the hypodermal cells (fig. 20) occurred in *Allium*. The enlargement, however, is general and not limited to regions over the protoxylem points, as was described by BURSTRÖM (7) for wheat.

Louis XIV *Tulipa* shows relatively little response to treatment. A very slight swelling of the root tip occurs, caused by limited enlargement of the cortical cells, which in the meristematic region show numerous divisions, at first radial and later both tangential and diagonal. The resulting daughter cells do not enlarge or show further development.

Cells of the pericycle undergo transverse division, and the daughter cells contain dense cytoplasm and have large nuclei, giving them the appearance of meristematic cells; but no proliferation

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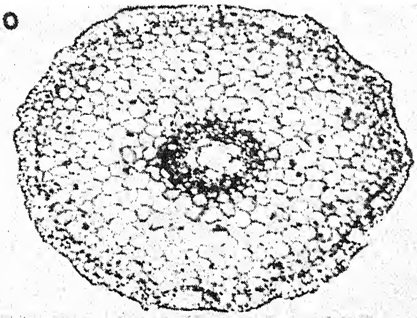

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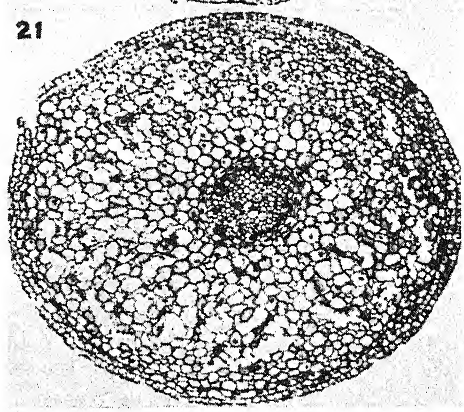

FIGS. 20, 21.—Fig. 20, *Allium* root, 48 hours after treatment with naphthyl acetamide, showing some enlargement of hypodermal cells. Fig. 21, *Narcissus*, 96 hours, showing disruption of cortex and compact layer of cells around periphery.

occurs and no root primordia are initiated. Mature cortical cells are stimulated to divide, and a number were observed in various stages of mitosis.

Within 48 hours after treatment, roots of *Narcissus* show prominent swellings a short distance above the tips, although pronounced enlargement does not always occur (fig. 15). The tumors result from cell enlargement only, involving chiefly the inner cortical cells—the peripheral cells remaining normal size. Continued enlargement of the cortical cells results in the formation of large schizogenous air spaces (fig. 21). Pericyclic cells show none of the activity shown by *Allium* and *Tulipa*, and no root primordia are initiated.

TRYPTOPHANE.—Roots were exposed to a 0.002% solution for 48 hours, after which the bulbs were returned to a balanced nutrient solution. Roots were killed at regular intervals from 24 to 192 hours after treatment.

Roots of *Allium* show little morphological change after treatment with tryptophane. No terminal swelling occurs, and there is only slight effect on cell polarity, since almost all mitotic figures are oriented normally. Up to 48 hours few of the young cortical cells show signs of recently completed division, although such divisions become more frequent later.

The endodermal cells divide tangentially, beginning about 72 hours after treatment, but become numerous only after 96 hours. The pericyclic cells, however, do not undergo the usual transverse divisions reported for the preceding substances. Pericyclic and endodermal cells gradually become less densely filled with cytoplasm as they mature. Neither initiation of root primordia nor proliferation of the pericycle takes place.

Mitosis occurs only in the meristematic region, the terminal 1.5–2 mm. of the root. In none of the material observed were the mature cells of the cortex stimulated to divide. Maturation of the tissues, particularly those of the stele, seems to occur more slowly in roots treated with tryptophane than in control material.

Roots of both tulips respond to tryptophane much as do those of *Allium*. Perhaps division in the endodermal and cortical cells occurs somewhat earlier in the John Ruskin. Approximately 72 hours after treatment, however, certain regions of the pericycle undergo transverse cell division, forming rows of short, almost isodiametric cells with dense cytoplasm. Tangential divisions take place in these cells but never become abundant. These responses occur at some distance proximal to the root tip.

The pericycle continues development from 96 to 192 hours after treatment, but the development is limited to definite regions. At first there is a mass of meristematic cells which shows no definite organization, but later there is a certain amount of organization. In a few instances the development of the proliferation is inward, resulting in some displacement of the vascular elements. Some cells of the proliferation take on the structure and appearance of wound tracheids, and it is possible that this leads to the formation of vascular tissue within the proliferation and the formation of vascular connections with the xylem of the root.

Narcissus roots develop slight spindle-shaped swellings near the tips, increasing the root diameter by about one-half. Cell polarity in the meristematic region is unaffected by treatment, and practically all the divisions are transverse. Pericyclic cells lose their dense cytoplasm in the elongation and maturation regions, and few radial and tangential divisions

occur. Mitosis is not promoted in the cells of mature tissues.

Discussion

The responses of these roots to treatment with the various growth-regulating substances extend, and to some extent agree with, the histological observations of LEVAN (17) and the general observations of BORGSTROM (6), GAVAUDAN (10), LANE (16), and others. Formation of terminal tumors on treated roots confirms the discussion and the illustrations presented by BORGSTROM. However, some of the data are not in agreement with the results of LEVAN and BURSTRÖM. The latter states that the rate of mitosis (in roots of wheat) is unchanged by the addition of heteroauxin (indole(3) acetic acid), but that such treatment does exert an influence on the direction of cell divisions, some being changed from transverse to radial or tangential; and thus an increase in the number of rows of cells is brought about, giving an increased root diameter. LEVAN states that "it will be seen from the longitudinal section that a larger number of cell rows is present than normally. This is due to the fact that the meristematic cortical cells under the influence of the growth substances start numerous mitoses. These mitoses are oriented in the transverse axis of the root, tangentially or radially." He then states that such divisions are not a necessary condition for the subsequent swelling of the cells, and that frequently the divisions are lacking.

In the present investigation there was no indication of increase in the number of vertical rows of cortical cells in the transition from the normal to the enlarged regions of the roots (figs. 2, 3, 11, 14, and 15), and few cases of cell division in any plane were observed in the cortical cells of the tumors. Divisions in many planes frequently were seen in the immature cortex below the tumor, however, and mitosis in mature cortical cells above the tumor was not uncommon. It is evident that treatment does affect cell polarity, for irregular divisions in the root tips did not occur in control material.

BURSTRÖM also described a hypertrophy of the hypodermal cells over the protoxylem strands in the mature region of roots of wheat seedlings, the enlargement becoming so extensive as to rupture the epidermis. Some hypertrophy of the hypodermis may occur in roots of *Narcissus* following various treatments, but it is always general and never very pronounced; and its occurrence is erratic. A similar condition occurred in the roots of *Allium* (fig. 20).

KRAUS and co-workers (14) have reported that in stems of Red Kidney bean treated with 3% indoleacetic acid in lanolin there is acceleration of mitoses, so that many cells become multinucleate. This condition was not found in root tumors of any of the plants investigated. In many instances, and in all roots studied, there were cells which appeared to be binucleate; but more critical observation showed that the nuclei were on entirely different focal levels, and almost invariably a film of cytoplasm and a cell wall lay between them. Longitudinal sections of roots frequently showed the nucleus of one cell resting against the lower wall, while the nucleus of the cell below lay against the upper wall. If observed in transverse section it might easily be interpreted as a cell containing two nuclei.

The ideas of the relationship between nuclear size and ploidy advanced by BERGER (3) cannot be taken too literally in studying an entire root section, although it probably is true that within the cells of any specific tissue such a rela-

tionship exists. For example, in a transverse section of a root the nuclei of the cortical cells are fairly large and prominent, but nuclei of the cells of the stele may be only one-fourth or one-eighth as large. If the same material is examined in longitudinal section, the nuclei of the cortical cells will be spherical or nearly so, while those in the stele are so elongated in some cases as to appear vermiform. Nuclear volume, however, may be essentially the same.

Marmer (19) has reported that the main or "primary" roots of wheat seedlings treated with indoleacetic, indolebutyric, or indolepropionic acid showed thickening, flattening, curling, and excessive root-hair formation at pH 4.6. In my investigations, root-hair formation was observed only after 120 hours or more in nutrient solution subsequent to treatment. The pH of the nutrient solution was approximately 5.5 at the time the bulbs were placed in it after treatment; and while no attempt was made to confirm this fact, it is probable that the physiological activity of the immersed roots resulted in the shifting of the pH to a point where root-hair formation was encouraged. An investigation of this phase of the work, using controlled pH, might be of interest.

That mitosis appears to be normal in those regions of *Lilium* stems showing response to treatment with indoleacetic has been reported by Beal (1). He found the diploid chromosome number to be present without exception. This is in agreement with these observations of roots of *Allium*, *Tulipa*, and *Narcissus* treated with indoleacetic acid and other growth substances. In the root primordia formed in *Allium* roots many mitotic figures were observed; and as far as could be determined, all nuclei had the diploid number of chromosomes.

Levan found that normal division occurred in most meristematic cells of *Allium*, but that some developments in the enlarged cortical tissues were of particular interest, among these being the occurrence of mitosis as far as 3 mm. from the root apex. Levan also found that while cells at the apex showed a normal diploid chromosome number, the majority of those farther back showed tetraploidy, and many of those still farther back showed octoploidy. In *Tulipa*, *Allium*, and *Narcissus*, mitosis occurred as far as 10–16 mm. from the root apex, far beyond the range of the terminal tumor; but no increase in chromosome number was observed in either *Tulipa* or *Narcissus*. Cells with 3n and 4n chromosomes were observed in *Allium*, but the number was sufficiently small to lie within the range of the normal occurrence of polyploid cells. As stated before, mitosis in the enlarged cortical cells was exceedingly rare.

Summary

1. Bulbs of *Allium cepa*, *Tulipa* (vars. John Ruskin and Louis XIV), and *Narcissus* (var. Paper White) were rooted and treated with dilute solutions of various growth-regulating substances.

2. Roots of *Allium* and *Narcissus* responded quickly to treatment with all substances except tryptophane by the formation of more-or-less globular terminal tumors. Tumors induced by tryptophane generally were longer, more slender, and somewhat more spindle-shaped. Roots of *Tulipa* showed little enlargement. Root-tip enlargement, where present, was due to an increase in the size of the cortical cells and not to an increase in the number of their rows.

3. Treatment resulted in the slowing down or complete cessation of root elongation. In some cases the effect was only

temporary, but in others it was permanent.

4. Histological responses occurred first in the meristematic region, chiefly in the cortex, where cells underwent division in all planes.

5. Division in the enlarged cortical cells was rare; but mature cortical cells above the tumors and as far as 16 mm. above the root apex were stimulated to divide. Such divisions generally were typical and in a transverse plane.

6. Pericyclic cells first responded by transverse divisions, forming rows of isodiametric cells with dense cytoplasm and large nuclei. These cells later divided radially and tangentially, resulting in considerable proliferation.

7. Proliferation did not occur in roots of *Narcissus*. Radial and tangential divisions occurred in the endodermal cells, but no true proliferation was apparent.

8. Proliferation of pericycle and development of numerous root primordia occurred in roots of *Allium*. Proliferations also occurred in both varieties of *Tulipa*, but the formation of definite, organized root primordia was not observed.

9. A few polyploid cells were seen in *Allium* roots, but none was found in either *Tulipa* or *Narcissus*.

10. Binucleate or multinucleate cells were not observed in any root subjected to any treatment.

The writer acknowledges with sincere thanks the advice and aid given by Dr. J. M. Beal of the University of Chicago during the course of this investigation.

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Zellwood, Florida

LITERATURE CITED

1. Beal, J. M., Histological responses of three species of *Lilium* to indoleacetic acid. Bot. Gaz. 99:881–911. 1938.
2. ———, Effects of indoleacetic acid on thin sections and detached segments of the second internode of the bean. Bot. Gaz. 102:366–372. 1940.
3. Berger, C. A., Some criteria for judging the degree of polyploidy of cells in the resting stage. Amer. Nat. 75:93–96. 1941.
4. Borthwick, H. A., Hamner, K. C., and Parker, M. W., Histological and microchemical studies of the reactions of tomato plants to indoleacetic acid. Bot. Gaz. 98:491–519. 1937.
5. Bonner, James, and Koepfli, J. B., Inhibition of root growth by auxins. Amer. Jour. Bot. 26:557–565. 1939.
6. Borgstrom, Georg, Influence of growth-promoting chemicals on roots of *Allium*. Botan. Notiser 1939:207–220. 1939.
7. Burström, Hans, Influence of heteroauxin on cell growth. Ann. Agr. Col. Sweden 10. 1942.
8. Cooper, W. C., Hormones and root formation. Bot. Gaz. 99:599–614. 1938.
9. Dermen, Haig, Intranuclear polyploidy in bean induced by naphthaleneacetic acid. Jour. Hered. 32:133–138. 1941.
10. Gavaudan, P., and Gavaudan, N., Tumefaction des racines par les substances modificatrices de la caryocinese. Compt. Rend. Séances Soc. Biol. 131 (16):168–171. 1939.
11. Hamner, K. C., Histological responses of *Mirabilis jalapa* to indoleacetic acid. Bot. Gaz. 99:912–954. 1938.
12. Harrison, B. F., Histological responses of *Iresine lindenii* to indoleacetic acid. Bot. Gaz. 99:301–338. 1938.
13. Kraus, E. J., Histological responses of bean plants to l-tryptophane. Bot. Gaz. 102:602–622. 1941.
14. Kraus, E. J., Brown, Nellie A., and Hamner, K. C., Histological reactions of bean plants to indoleacetic acid. Bot. Gaz. 98:370–420. 1936.
15 Kraus, E. J., and Mitchell, J. W., Histological and physiological responses of bean plants to alpha naphthalene acetamide. Bot. Gaz. 101:204–225. 1939.
16. Lane, R. H., Inhibition of roots by growth hormones. Amer. Jour. Bot. 23:532–534. 1936.
17. Levan, Albert, Cytological phenomena connected with the root swelling caused by growth substances. Hereditas 25:87–96. 1939.
18. Macht, D. I., and Grumbein, Mary L., Influence of indoleacetic, indolebutyric, and naphthaleneacetic acids on roots of *Lupinus albus* seedlings. Amer. Jour. Bot. 24:457–460. 1937.
19. Marmer, Dina R., Growth of wheat seedlings in solutions containing chemical growth substances. Amer. Jour. Bot. 24:139–145. 1937.
20. Thimann, K. V., Auxins and the growth of roots. Amer Jour. Bot. 23:561–569. 1936.
21. Zimmerman, P. W., and Hitchcock, A. E., Response of roots to "root-forming" substances. Contr. Boyce Thompson Inst. 7:439–445. 1935.

ENHANCED AUXINIC ACTIVITY OF TOMATO TISSUES IN PRESENCE OF L-TRYPTOPHANE[1]

CONTRIBUTIONS FROM THE HULL BOTANICAL LABORATORY 558

GEORGE K. K. LINK AND VIRGINIA EGGERS

By means of procedures reported earlier (3, 4, 8), the effect of the addition of l-tryptophane[2] on the auxinic activity of lyophilized and fresh tomato tissues was tested. Extracts of tomato hypocotyls were used as controls and compared with extracts of hypocotyls inoculated with *Phytomonas tumefaciens.*

Dry weights of both control and inoculated hypocotyls (table 1) equal about 10% of their wet weights. Auxinic activity of the residue of each sample is expressed in an arbitrary unit, calculated on the basis of 1 gm. dry weight in 1 cc. of agar, and an average sensitivity of *Avena* coleoptiles of 16° curvature for 30 γ indoleacetic acid per liter of agar (3, 4).

TABLE 1

SAMPLES USED IN EXPERIMENTS 1–4 AND HISTORY OF THEIR SOURCES

Seed planted	Hypocotyls inoculated	Hypocotyls collected, lyophilized, and powdered	Hypocotyls collected and used fresh
3/7/43....	4/8/43	5/7/43 (Exp. 2)	....
5/8/43....	6/1/43	6/28/43 (Exps. 1, 3)	....
8/24/43...	9/14/43	....	10/6/43 (Exp. 4)

Procedures used in experiments 1–4 are given in the legends of the tables.

An identical experiment was carried out with powder of the 5/4/43 lot. Parallel results were obtained, but the auxinic activity of these samples was greater, showing that the cultural and nutritional histories of the plant affect the auxinic activity of its extracts.

The results of experiment 4 (table 5) corroborate earlier findings (3, 4) that extracts of crown-gall tissues are more active auxinically than those of healthy tomato tissues. They also show that, while extracts of lyophilized tissues are not so active as those of fresh tissues, the findings for lyophilized tissues are applicable to fresh tissues (3).

EXPERIMENT 1: TABLE 2

AUXINIC ACTIVITY, IN UNITS, OF (1) RESIDUES OF EXTRACTS A, B, C OF LYOPHILIZED POWDER (6/28/43) IN 200 CC. OF ETHER, AND OF (2) 1 MG. OF TRYPTOPHANE (D) DISSOLVED IN 200 CC. OF ETHER AFTER STANDING IN THE DARK AT 4° C. FOR 7 DAYS. RESIDUES TAKEN UP IN AGAR AT 60° C.

Source of residue	Activity
a. 0.4 gm. powder......................	108.0
b. 0.4 gm. powder, plus 1 mg. tryptophane added to residue before taking up in agar............................	129.6
c. 0.4 gm. powder and 1 mg. tryptophane placed simultaneously in ether......	662.4
d. 1.0 mg. tryptophane in 200 cc. ether...	4.8

The findings of experiment 1 (table 2d) indicate either that the tryptophane used contained some auxinically active impurities or that a slight amount of it is converted into active material by aqueous ether or in the course of being taken up in warm agar (1, 7). The latter is indicated, since experiment 1 (table 2b) shows an increase in auxinic activity when tryptophane is added to warm agar containing residues of plant extracts. This, together with the general data of all experiments, indicates that results obtained by the standard methods of extraction and of taking up of residues in

[1] This work was supported in part by a grant from the Wallace C. and Clara A. Abbott Memorial Fund of the University of Chicago.

[2] We are indebted to our colleague, Professor EARL EVANS of the Department of Biochemistry, for a gift of l-tryptophane prepared in his laboratory. Eastman's l-tryptophane was also used.

warm agar are measures, not only of the auxinically active substances present in the sample at the onset of extraction but also of the auxinically active materials obtained from tryptophane by conversion. Results of experiment 3 (table 4) show that time is a factor in the conversion of precursors to active material,

EXPERIMENT 2: TABLE 3

AUXINIC ACTIVITY, IN UNITS, OF (1) RESIDUES OF EXTRACTS A, B, C, D OF LYOPHILIZED POWDER (5/4/43) IN 100 CC. OF ETHER AFTER STANDING IN DARK FOR 12 HOURS, TAKEN UP IN AGAR AT 60° C., AND OF (2) AGAR BLOCKS, 6 CU. MM., AFTER STANDING 2 HOURS IN 0.132 CC. H_2O CONTAINING 2 MG. TRYPTOPHANE (E)

Source of residue	Activity
a. 0.2 gm. powder..........................	165.0
b. 0.2 gm. powder boiled 15 minutes in 10 cc. H_2O prior to placing in ether, plus residue of ether extract of H_2O used in boiling the powder.*..............	85.8
c. 0.2 gm. powder, plus 0.5 mg. tryptophane placed in ether with the powder	176.0
d. 0.2 gm. powder, boiled 15 minutes in 10 cc. H_2O prior to placing in ether, plus 0.5 mg. tryptophane placed in ether with the powder, plus residue of ether extract of H_2O used in boiling the powder*.........................	67.65
e. 2.0 mg. tryptophane dissolved in 0.132 cc. cold H_2O and taken up by 12 cubes of agar, each 6 cu. mm.............	0.0

* In similar tests, residue of ether extract of this water generally is auxinically negative.

and that the activity obtained at any moment is not an index of a definite fraction of the total potential auxinic activity of the sample.

The results of experiment 2 (table 3e) indicate that tryptophane either does not penetrate the epidermis of the upper part of the *Avena* coleoptile or is not auxinic for its tissues. They may indicate either that these tissues, unlike those of tomato, and possibly of bean (2), are not able to bring about conversion of tryptophane, or that the 100 minutes during which the agar blocks are in contact with the tissues of the coleoptile are not sufficient time for a measurable conversion.

EXPERIMENT 3: TABLE 4

AUXINIC ACTIVITY, IN UNITS, OF RESIDUES OF EXTRACTS OF 0.4 GM. LYOPHILIZED POWDER (6/28/43), WITH AND WITHOUT 1 MG. TRYPTOPHANE, IN 200 CC. ETHER, AFTER STANDING IN DARK AT 4° C. FOR 12 HOURS, 6 DAYS, AND 13½ DAYS. RESIDUES TAKEN UP IN AGAR AT 60° C.

Extraction time	Activity	
	Without tryptophane	With tryptophane
12 hours.........	54.0	85.0
6 days..........	60.0	640.0
7 days..........	20.0	910.0
Total 13½ days	134.0	1635.0

EXPERIMENT 4: TABLE 5

AUXINIC ACTIVITY, IN UNITS, OF RESIDUES OF EXTRACTS OF 5.0 GM. OF FRESH TOMATO HYPOCOTYL AND OF INOCULATED HYPOCOTYLS (10/6/43), SLICED 1–2 MM. THICK, PLACED IN 50 CC. ETHER, WITH AND WITHOUT 1 MG. TRYPTOPHANE, AFTER STANDING IN DARK AT 4° C. FOR 12 HOURS. RESIDUES TAKEN UP IN AGAR AT 60° C.

	Noninoculated hypocotyl		Inoculated hypocotyl	
	Without added tryptophane	With added tryptophane	Without added tryptophane	With added tryptophane
Activity....	32.3	256.0	836.9	1390.7

The findings of experiment 2 (table 3a–d) indicate that the conversion of tryptophane is brought about by enzymes of the tomato tissues. Tryptophane apparently is a possible precursor of auxinically active materials (5, 7).[3]

[3] A recent paper indicates that tryptophane is not the precursor of "auxin" in corn and wheat

The rate and amount of conversion during extraction is one limiting factor in the activity obtained. Any one of the steps whereby plant proteins are converted into polypeptides, polypeptides into tryptophane, and tryptophane into active material (1, 3, 5, 6, 7, 9), as well as the rate at which tryptophane is synthesized in the plant, may be limiting as to the amount of active material present at any moment.

Accordingly, results obtained with the coleoptile test are indirectly, in part at least, an index either of the rate of the activity of these enzymes or of the concentrations of the enzymes involved, or of both. At any moment, therefore, crown-gall tissue either contains more of these enzymes than do normal tissues, or its enzymes are more active. Extractions extending through 2 years often do not exhaust the precursors of the active materials of these tissues, showing that generally amounts of the precursor materials are not the limiting factors.

So far as understanding regulation of growth by auxinically active substances is concerned, the critical problem deals not merely with the concentration of these substances and of their precursors. The heart of the problem is this: that in the presence of an excess of precursor, and with the proper enzymes actually or potentially available, conversion at any moment proceeds at a rate to yield that concentration of active substances which effects the amount and kind of growth and development realized. Auxinically active substances probably are agents of growth regulation, but the regulation itself apparently is that event in the plant which determines the time and place of appearance of these agents as well as their concentration.

UNIVERSITY OF CHICAGO

LITERATURE CITED

1. GORDON, S. A., and WILDMAN, S. G., The conversion of tryptophane to a plant growth substance by conditions of mild alkalinity. Jour. Biol. Chem. **147**:389–398. 1943.
2. KRAUS, E. J., Histological reactions of bean plants to l-tryptophane. BOT. GAZ. **102**:602–622. 1941.
3. LINK, G. K. K., EGGERS, VIRGINIA, and MOULTON, J. E., Use of frozen vacuum-dried material in auxin and other chemical analysis of plant organs: Its extraction with dry ether. BOT. GAZ. **102**:590–601. 1941.
4. LINK, G. K. K., and EGGERS, VIRGINIA, Hyperauxiny in crown gall of tomato. BOT. GAZ. **103**: 87–106. 1941.
5. SKOOG, F., A deseeded *Avena* test method for small amounts of auxin and auxin precursors. Jour. Gen. Physiol. **20**:311–334. 1937.
6. SKOOG, F., and THIMANN, K. V., Enzymatic liberation of auxin from plant tissues. Science **92**:64. 1940.
7. STEWART, W. S., Effectiveness of tryptophane mixtures as growth regulators. BOT. GAZ. **102**: 801–805. 1941.
8. WENT, F. W., and THIMANN, K. V., Phytohormones. New York. 1937.
9. WILDMAN, S. G., and GORDON, S. A., The release of auxin from isolated leaf proteins of spinach by enzymes. Proc. Nat. Acad. Sci. **28**:217. 1942.

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grains (AVERY, G. S., and BERGER, J., Tryptophane and phytohormone precursors. Science **98**:513–515. 1943).

FREQUENCY OF POLYEMBRYONY IN FRAXINUS SEEDS

G. P. STEINBAUER

During the course of some experiments on delayed germination of *Fraxinus* seeds (1), it was noted that the individual samaras occasionally gave rise to two or more seedlings. At first it was thought that this might be accounted for by the presence of more than one seed per fruit. Closer examination indicated that while this was usually true, there were instances in which twin embryos developed from an individual seed. Polyembryony is infrequent in angiosperms, although it may be common in certain groups, such as *Citrus* and *Poa*.

Table 1 indicates the frequency of the phenomenon as observed in certain lots of *Fraxinus* seeds. The data are based on soaked samaras of *F. americana* L., *F. pennsylvanica* Marsh., *F. nigra* Marsh., and *F. velutina* Torr. var. *toumeyi* (Britt.).

The samara of *Fraxinus* is typically a one-seeded fruit with one embryo per seed. The frequency of two seeds per fruit varied from 0.71 per cent in *F. nigra* to 7.00 per cent in *F. velutina*.

The exact origin of the plural embryos is not known. Since many of the reported instances are based on seedling production, the origin of the multiple embryos is often obscure. WEBBER (3) points out that since neither the production of plural eggs nor the liberation of multiple sperms in the megagametophyte is characteristic of angiosperms, it is doubtful whether simple polyembryony occurs. It is suggested that the more likely origin of multiple embryos is from synergids or antipodal cells. Although most of the twin embryos in *Fraxinus* lie parallel to the long axis of the seed, with radicles near the micropyle, in several instances the embryos were oriented opposite each other, one with the radicle adjacent to the micropyle and the other with it at the antipodal end of the megagametophyte. This may suggest the origin of extra embryos from antipodal cells, although their possible origin from supernumerary eggs, from synergids, or from sporophytic budding is as likely.

Polyembryony may occur in *F. pennsylvanica* despite the absence of an observed case. In several plant groups there is a high mortality among multiple embryos during early development. In certain gymnosperms as many as 200 embryos may originate in the seed although only one or a very few survive. TRAUB (2) has shown that the survival of multiple embryos in *Citrus* is dependent not only on the inherent tendencies but also on such factors as available food supply, temperature, etc. It is possible that such factors may also operate to determine embryo survival in *Fraxinus* and other seeds.

TABLE 1

NUMBER OF SEEDS PER FRUIT AND EMBRYOS PER SEED IN FOUR SPECIES OF FRAXINUS

Species	No. of fruits examined	Percentage: Fruits with 1 seed	Fruits with 2 seeds	Seeds with twin embryos
F. nigra	2773	99.29	0.71	0.11
F. americana	2610	98.90	1.10	0.11
F. pennsylvanica	3754	99.02	0.98	0.00
F. velutina	2293	93.00	7.00	0.13

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LITERATURE CITED

1. STEINBAUER, G. P., Dormancy and germination of *Fraxinus* seeds. Plant Physiol. 12:813–824. 1937.
2. TRAUB, H. P., Artificial control of nucellar embryony in *Citrus*. Science 83:165–166. 1938.
3. WEBBER, J. M., Polyembryony. Bot. Rev. 6: 575–598. 1940.

CURRENT LITERATURE

Edible Wild Plants of Eastern North America. By MERRITT LYNDON FERNALD and ALFRED CHARLES KINSEY. Cornwall-on-Hudson: Idlewild Press, 1943. Pp. xiv+452. $3.00.

A book such as this is of great value at any time, particularly the present. It is very comprehensive, not only in the great number of forms given, but in the clear, adequate descriptions of them, their habitat and range, and how they may best be utilized. The chapter on Poisonous Flowering Plants Likely to be Mistaken for Edible Species is a valuable section. Like the rest of the book, its statements are positive and clear.

Many are familiar with various wild plants useful as food. Nuts, berries, pot-herbs, and salad plants are common in modern diets, although the degree to which some of them, either directly from the wild or under partial or intensive cultivation, are used, is often not realized. They are now taken for granted. The authors list dozens more which are useful, or could be made so. One is fascinated to turn the pages and find again and again familiar subjects listed among the wild species.

The imagination is stimulated to find suggested purees and soups from elder pith, flowering spikes of cat-tails, daylily buds, and dewberries; starchy foods from lichens, lily bulbs, white waterlily, evening-primrose; cat-tail pollen, clover heads and seeds, inner bark of pine and hemlock and many daily acquaintances; pot-herbs and salad plants of many fairly familiar and less familiar kinds, including clover, chickweed, plantain, mallow, yucca flowers, burdock and Scotch thistle; nibbles and relishes including dried ginger, trailing arbutus flowers, rose petals, alder buds and bark; pickles and condiments in great array; drinks from slippery elm, sassafras, holly leaves, elder flowers, persimmon seeds, dandelion root, chinquapin, and many familiar berries and roots; sugars, confections, preserves from many less usual species and others now taken for granted, such as strawberries, raspberries, cranberries, walnuts, pecans, and many another. In addition, there are lists covering fruits suitable for drying, oils and butters, chewing gums and substitutes, and emergency foods.

Seldom indeed has so comprehensive and authoritative a book dealing with useful wild food plants been based on such outstanding authority.—E. J. KRAUS.

The American Land: Its History and Its Uses. By WILLIAM R. VAN DERSAL. New York: Oxford University Press, 1943. Pp. vii+xvi, 215. $3.75.

The work treats very briefly and in a popular manner many of the uses and abuses of land devoted to the production of food and feed crops. The final chapter offers suggestions for greater conservation of soil resources and possible methods for increasing crop production.

Various crops are mentioned, but treatment is scarcely more than a list of them, except perhaps Indian corn. There are many excellent clear, often striking, photographs of definite value to the casual reader, for whom apparently the work is primarily intended.

The most significant and pertinent statements in the entire book are found in the last paragraph of the author's preface: "Finally, to all those farmers around the country with whom I have talked and through whose eyes I have seen much more than I thought possible, I offer my appreciation of their patience and my admiration for their wisdom. Sometimes I think they know more about America than anyone else."

And plant problems as well.—E. J. KRAUS.

A Dictionary of the Fungi. By G. C. AINSWORTH and G. R. BISBY. Kew: The Imperial Mycological Institute, 1943. Pp. viii+359. $4.60.

An attempt is made to list alphabetically all the generic names of fungi (Eumycetes and Myxothallophyta) that have been in use to the end of 1939, including the systematic position, distribution, and number of species of each genus. There are also short accounts of the chief families, orders, and classes of the fungi and of the bacteria and lichens; definitions of words used in mycology; the common and scientific names of important fungi; and other matters of interest, such as culture methods and media and short biographies of some of the pioneer workers in mycology. Finally, an appendix gives G. W. MARTIN'S *Key to the Families of the Fungi.*

The book represents a tremendous amount of work on the part of the authors, and it is highly recommended to all who are interested in the fungi.—J. M. BEAL.

Diseases and Pests of Ornamental Plants. By BERNARD O. DODGE and HAROLD W. RICKETT. Lancaster: Jaques Cattell Press, 1943. Pp. xi+638. $6.50.

Various treatises have been issued from time to time dealing with the pests which infest various crops of economic importance. The present volume is a comprehensive one concerning ornamental plants. Part I covers the general types of pests which are likely to be encountered, of animal, plant, or undetermined origin, and the principles involved in pest control. Part II is an extensive alphabetical list of ornamental plants, together with the pests affecting each, and methods of control.

The text is direct and clear, the many illustrations well done and to the point. It should prove a useful handbook and manual for those who attempt to grow ornamentals and to those called upon to furnish suggestions on pest control.—E. J. KRAUS.

Frontiers of Cytochemistry: The Physical and Chemical Organization of the Cytoplasm. Edited by

NORMAND L. HOERR. Lancaster: Jaques Cattell Press, 1943. Pp. 334. $3.50.

This volume is number X in a series of Biological Symposia. It is a tribute to the work of Emeritus Professor ROBERT R. BENSLEY, of the University of Chicago. Originally the papers were prepared for the celebration of the seventy-fifth anniversary of Dr. BENSLEY's birth and were presented before an audience of about 500 on November 13, 1942.

A brief appreciation of Dr. BENSLEY as a leader in research, dealing with the physical and chemical organization of protoplasmic constituents, is written by E. V. COWDREY. The published papers number fourteen, the first of which, by ARNOLD LAZAROW, is a summary of the work done in BENSLEY's laboratory during the last ten years. Many features of cellular organization and behavior are discussed in the several papers. The separation of protoplasm into its individual components, and the analysis of these components by physical and chemical means, form the central theme of the symposium. Although most of the work has been done on animal cells, it seems probable that the findings are fundamentally applicable to all protoplasm, with some variability in the quantitative amounts of the constituents present. The last paper in the volume is by Dr. BENSLEY, on the Chemistry of Cytoplasm.

The papers constitute a very valuable summary of progress in a difficult field and emphasize the importance of leadership such as Professor BENSLEY has been able to give in this field. An excellent portrait of Dr BENSLEY is appended.—C. A. SHULL.

Flora of Peru (Leguminosae). By J. F. MACBRIDE. Chicago: Field Mus. Nat. Hist. Bot. Ser. 13, 1943. Part 3, no. 1. Publ. 531. Pp. 1–507.

This is a conventional taxonomic treatment of the single family Leguminosae, with 18 genera of the Mimosoideae, 28 of the Caesalpinioideae, and 72 of the Papilionatae recognized as occurring in Peru. Keys to the genera and to the species are provided, with ample generic descriptions as well as descriptions of all recognized species. The synonymy is given for each species, and under each all Peruvian collections representing the entity are cited. Approximate extra-Peruvian distribution is indicated.—E. D. MERRILL.

Bibliography of References to the Literature on the Minor Elements and Their Relation to Plant and Animal Nutrition. New York: Chilean Nitrate Educational Bureau, Inc., 1943. Pp. 92.

The fourth supplement to the third edition of this bibliography continues the plan of arrangements of previous supplements. There are approximately 700 abstracts, dealing with more than half of the known elements and with more than 100 kinds of crop plants. The abstracts are arranged by elements, in alphabetic order, from aluminum to zinc and zirconium, with the rare earths and miscellaneous at the close.

The triple index, by elements, by botanical species, and by authors, leaves nothing to be desired. It is an important work, and a service which deserves the wholehearted appreciation of American scientists. It is especially useful to those who are engaged in mineral nutrition investigations.—C. A. SHULL.

The Food Resources of Africa. By THOMAS S. GITHENS and CARROLL E. WOOD, Jr. Philadelphia: University of Pennsylvania Press, 1943. Pp. 105. $1.50.

This brief treatise gives a general survey of Africa's agricultural resources. Based on the continent as a whole, statistics of production are given for its major geographical—and to a lesser extent its ecological—areas. These form a brief crop index of present production, utilization, and export of the principal food crops.—E. J. KRAUS.

ANATOMY AND SORUS DEVELOPMENT OF CAMPTOSORUS RHIZOPHYLLUS

BARBARA F. PALSER

Introduction

Camptosorus rhizophyllus (L.) Link is a native of eastern and central United States and Canada. There is one other species in the genus, *C. sibiricus* Rupr., a native of Siberia, China, and Japan. *C. rhizophyllus* grows in shady places, particularly on calcareous rocks, where the plants may form dense mats of overlapping fronds.

The general opinion that *C. rhizophyllus* is found chiefly on calcareous rocks led WHERRY (45, 46) to investigate whether the presence of this plant was diagnostic of such rock. In many regions he found the plant growing on rocks varying in lime content from 53.8% to less than 0.1%. The soils supporting its growth, however, are highly calcareous—having more lime than the average field soil—with reference to both total and soluble lime content of the soil. He concludes that this plant does not necessarily indicate the presence of calcareous rock but of calcareous soil.

C. rhizophyllus and *C. sibiricus* have had a varied history in classification, as is shown in CHRISTENSEN's Index (8): *Asplenium* by LINNAEUS, 1753 (28); *Camptosorus rhizophyllus* by LINK, 1833; *C. sibiricus* by RUPRECHT, 1845; *Scolopendrium* by ENDLICHER, HOOKER and BAKER (25), CHRIST (7), and DIELS (18); *Antigramma* by J. SMITH; and *Phyllitis* by O. KUNTZE. It is now separated generically from *Phyllitis*, the genus which at present includes all species of *Antigramma* and *Scolopendrium*. The resemblance between *Camptosorus* and *Phyllitis* is indicated by their history in classification. Both ferns have geminate sori on the lateral veins, with indusia opening toward each other.

The groups which offer the closest similarity to *Camptosorus* are the Asplenioids and Blechnoids because of the linear, superficial sori. CHRISTENSEN (9), basing his conclusions largely on habit, places *Camptosorus* and *Phyllitis* in the Asplenioids. BOWER (3, 4, 5) allies them to the Blechnoideae. In the Asplenioids the sori are on lateral veins, but the indusia all open in the same direction, or, as in *Diplazium*, away from each other. In the Blechnoids the sorus is a coenosorus or chain of sori, with the indusia opening toward the midrib. BOWER traces the formation of the soral type of arrangement in *Camptosorus* and *Phyllitis* through the breakdown of the coenosorus, as seen in *Blechnum punctulatum* var. *krebsii*, in which the coenosorus has arched outward and been partially disrupted in those leaves changing from a narrow highly pinnate form toward a broad expanse. *Camptosorus* shows gradation from irregularly arranged sori at the base of the leaf to a sorus reminiscent of the Blechnoid coenosorus at the caudate tip of the leaf.

Certain aspects in the life history of *C. rhizophyllus* have been investigated by various workers: soral arrangement by the early taxonomists, by BOWER (3, 4, 5), and by CHRISTENSEN (9); vegetative reproduction by GOEBEL (20),

KUPPER (27), MCVEIGH (29, 30), and YARBROUGH (48); the possibility of hybridization between *C. rhizophyllus* and species of *Asplenium* by BRAUN (6), MAXON (31), HOYT (26), COULTER (12), DAVENPORT (13, 14), COPELAND (10), UNDERWOOD (44), SLOSSON (42), and BENEDICT (2); development of the prothalli by PICKETT (38) and WUIST (47); the resistance of the prothalli to desiccation by PICKETT (37, 39); and the type of stomata by COPELAND (11).

With the discussion of vegetative reproduction and of hybridization in *Camptosorus*, some mention of anatomical structure is found. YARBROUGH (48) describes briefly the rhizome apex and leaf primordia; MCVEIGH (30), the apical growth of leaves; and SLOSSON (42), the dermal appendages. HAYATA (22, 23) records observations on the anatomy of the stem of *C. sibiricus*. The stele is a radial dictyostele, with a single leaf trace bundle. A single root trace branches from the meristele just below the origin of the leaf trace strand.

The present investigation of the details of the anatomy of rhizome, leaf, and root, and of the early development of the sorus of *C. rhizophyllus* supplements the few anatomical facts already known about this species. Relationships between *Camptosorus* and other genera are elucidated when such anatomical details are compared.

Material and methods

Camptosorus rhizophyllus was collected on the eastern slope of Mount Toby, Massachusetts, during 1939. Leaves were killed in Navashin's solution, rhizomes in a chromo-acetic solution containing 1% chromic acid and 3% acetic acid. Rhizomes were treated with 10% hydrofluoric acid before imbedding. All material was imbedded in paraffin and sectioned at 7–10 μ. Stains used were Heidenhain's iron-alum haematoxylin counterstained with safranin, safranin-fast green, and safranin-gentian violet.

Diagrams of every tenth section of a cross-sectional series of the rhizome were made, and from these a clay model of the vascular system was built up.

Observations

HABIT.—The stem of *C. rhizophyllus* is short, upright, and gives rise to the simple leaves in spiral succession. The leaves vary somewhat but in general are small and often gradually narrowed from a cordate or auricled base to a long slender acuminate tip which may root (fig. 1). Overall length of the blade may be 4–14 cm. The petiole, 1–6 cm. in length, is green, slightly winged for some distance below the blade, and bears dark brown acuminate scales at the base. The venation is partially reticulate, but toward the margin and the tip of the blade the veins are free.

The sori are oblong or linear and irregularly scattered on the reticulated veins; those next to the midrib are single, while the outer ones are inclined to approximate in pairs, so that the two indusia open face to face, or become confluent at their ends, forming a V-shaped sorus (fig. 1).

RHIZOME.—As determined from both sections and surface views, the growing tip of the rhizome is protected by many scales and by the surrounding leaf bases. The apex itself is flattened and has only a suggestion of conical configuration (fig. 3). An apical cell with three cutting faces is present (fig. 2). Leaf primordia are seen close to the apical cell and are formed from segments of it (figs. 2, 3).

In studying the cellular structure of the rhizome apex, occasionally one or two spiral tracheids may be seen ma-

turing just a short distance behind the growing region. In many cases, however, the first tracheids to mature are scalariform but without strong lignification. The scarcity of spiral or annular tracheids may be related to the slow growth of the rhizome. The protoxylem cells are few and scattered irregularly through the meristele. The metaxylem cells are scalariform, the first formed often being small in size. The first tissues of the stem to mature are the endodermis and phloem. The phloem cells are elongated, very small in cross section, and have slightly thickened walls. The endodermal cells are thin-walled, except for Caspary's strips on the radial walls.

The rhizome has an unperforated dictyostele, with curving meristeles bordering the leaf gaps (fig. 4). Cross sections of a mature rhizome show the number of meristeles to be ordinarily three, although two or four may be found (fig. 5*a–f*). There are two traces in each leaf base, arising on opposite sides of the leaf gap, which is formed by the division of a single meristele into two strands. Each strand may give rise to a leaf trace bundle immediately (fig. 5*e* top), while uniting with an adjacent meristele (fig. 5*e* bottom), or after uniting with an adjacent meristele. In general, one meristele gives off a leaf trace bundle before merging, while the other gives off one while merging—or after it has merged with an adjacent strand. The leaf trace bundles diverge from the meristeles on either side of the leaf gap just above its opening, but they do not always arise at the same level. The root trace associated with the leaf base usually arises from the meristele as it divides to form the leaf gap (fig. 4); occasionally it may arise from one of the meristeles shortly after the leaf gap is formed (fig. 4, *rt tr* 1).

Each meristele (fig. 6) is delimited from the cortex by a continuous endodermis. A layer of pericycle, one to two cells thick, lies between the endodermis and the phloem. The xylem, more or less

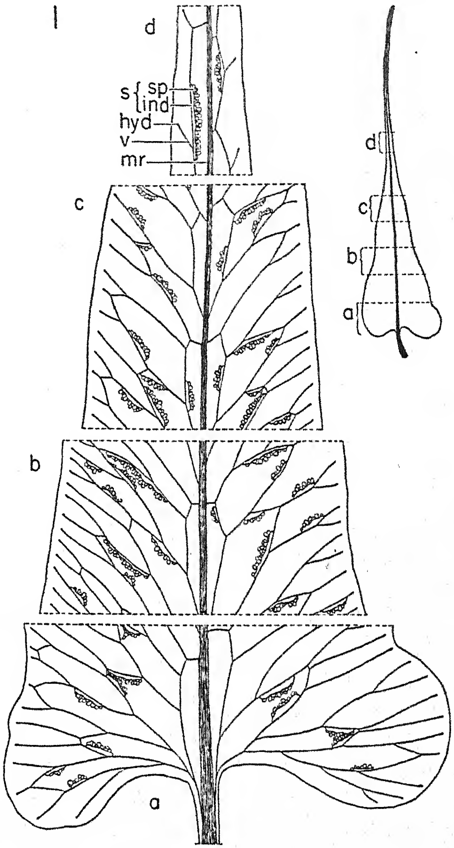
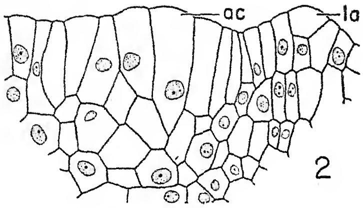
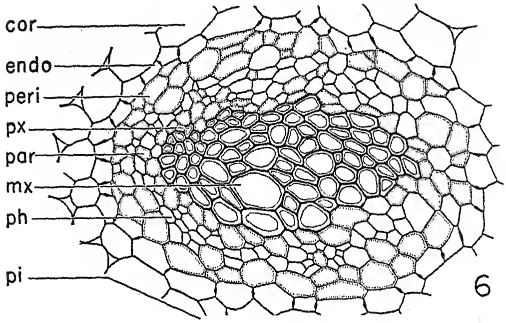
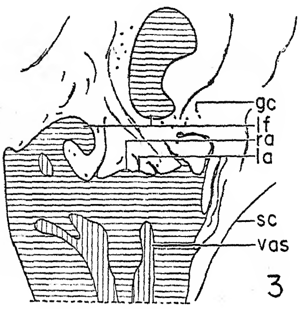


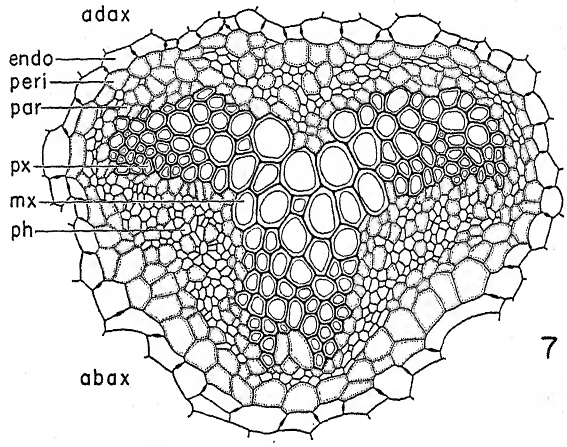
FIG. 1.—Diagram and partial detail of leaf: *s*, sorus; *sp*, sporangium; *ind*, indusium; *hyd*, hydathode; *v*, vein; *mr*, midrib.

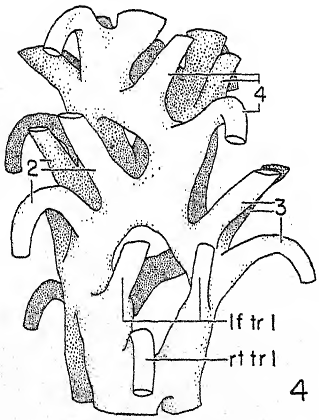
oval in cross section, is surrounded by parenchyma, and outside that by a layer of phloem which is not always continuous. The protoxylem cells are few and scattered and not noticeable in a mature meristele. Small metaxylem cells are found at the two ends of the oval core and to some extent along the cortical side. The larger metaxylem cells are toward the pith.

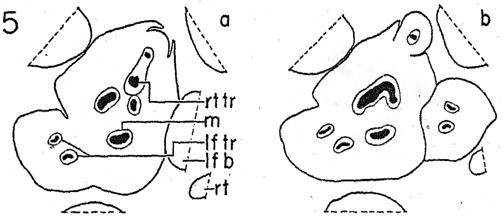


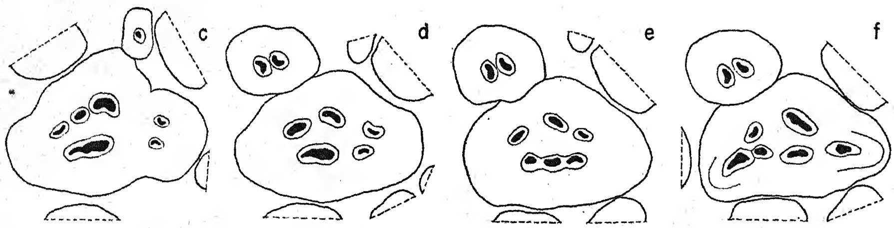












FIGS. 2–7.—Fig. 2, apex of rhizome: *ac*, apical cell; *la*, leaf apex. Fig. 3, diagram of longisection of rhizome through apex: *gc*, glandular cell; *lf*, young leaf; *ra*, rhizome apex; *sc*, scale; *vas*, vascular system. Fig. 4, reconstructed vascular system; *lf tr*, leaf trace; *rt tr*, root trace; in order of formation: 1, first; 2, fourth; 3, third; 4, sixth; opening of nine leaf gaps included in model. Fig. 5, cross sections of rhizome (*a–f*): *m*, meristele; *lf b*, leaf base; *rt*, root. Fig. 6, detail of meristele: *cor*, cortex; *endo*, endodermis; *peri*, pericycle; *px*, protoxylem; *par*, parenchyma; *mx*, metaxylem; *ph*, phloem; *pi*, pith. Fig. 7, detail of vascular bundle of upper petiole and midrib of leaf: *adax*, adaxial side of bundle; *abax*, abaxial side.

In cross section each vascular strand of the leaf trace is crescent-shaped, the concave side facing diagonally outward. Spiral tracheids, making up the protoxylem of the traces, are located at the tips of the crescent, but those first formed are one or two cells back from the tip on the convex surface. The larger metaxylem cells are found in the center of the convex curve, on the adaxial side of the bundle. In the rhizome and part way through the petiole the two strands remain distinct (fig. 8). As they approach the leaf blade, first the endodermis becomes continuous around the two bundles (fig. 9), then the phloem does not develop between the xylem tracts, and finally the latter become contiguous (fig. 10), in which condition the xylem is found in the midrib of the leaf. The merging reduces the number of protoxylem points to three as the two abaxial groups and part of the adjacent metaxylem approach each other and become indistinguishable, forming a trace V-shaped in cross section. The two strands do not completely lose their identity. Occasionally the adaxial tips are not completely joined, showing a slight tendency to form an X-shaped trace (fig. 7). Phloem is found in greatest amount in the concavities of the trace where it matures first, but it is also present around the whole trace in form of a few scattered cells.

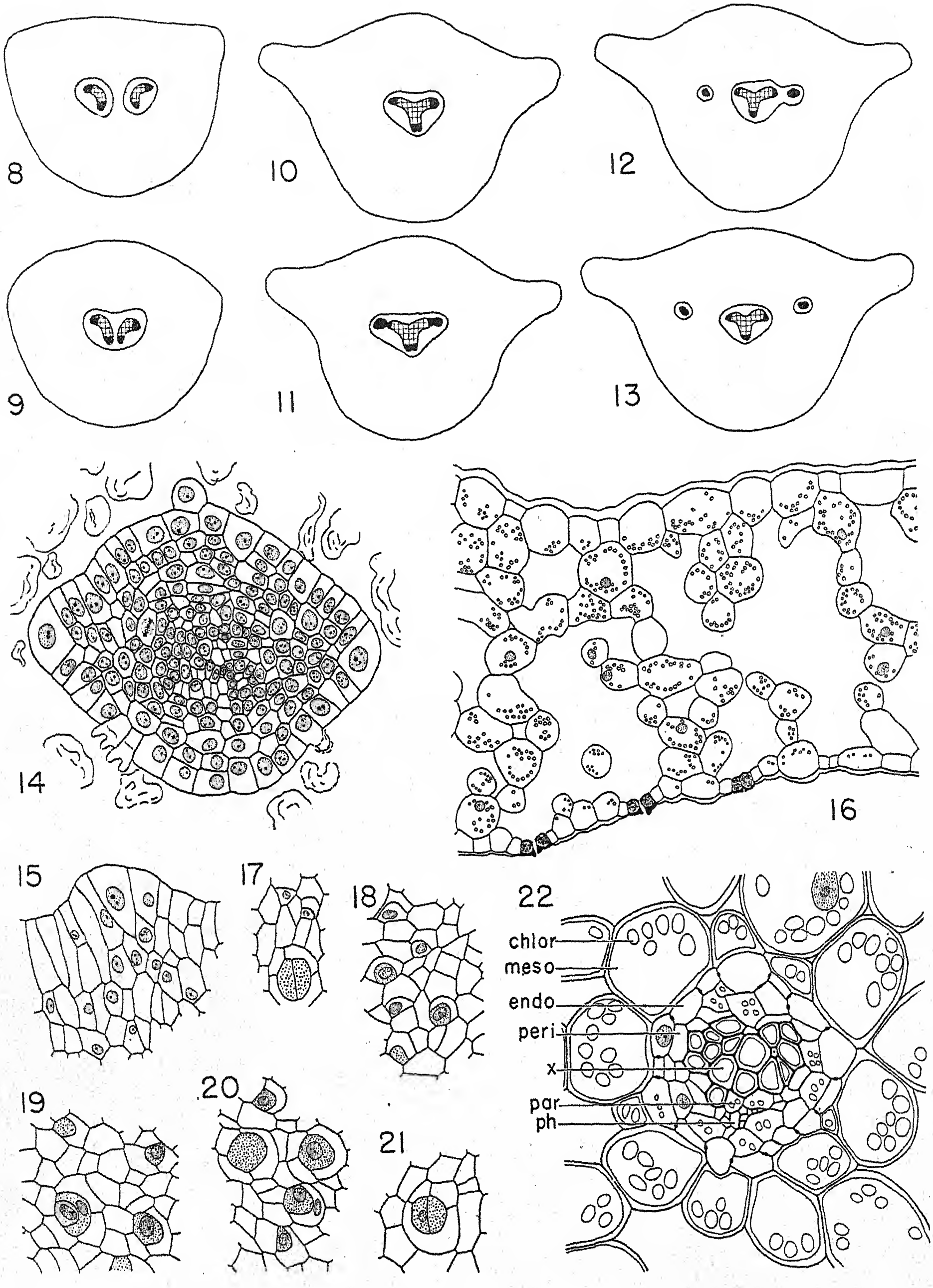
Although *Camptosorus* has a simple leaf, the origin of the first veins arising from the leaf trace in the petiole below the blade may be compared with that of pinna-traces of ferns with pinnate leaves. The adaxial corners of the xylem strand gradually diverge from the main strand, but the other tissues remain continuous for a longer time (figs. 11, 12). Each of the newly diverged lateral veins (fig. 13) is made up of a more or less flattened strand of xylem, with phloem largely on the abaxial side but with a few scattered cells on all sides. It is surrounded by a complete endodermis and pericycle.

Leaf.—The leaf arises about seven cells away from the apical cell of the rhizome. The first evidence of a leaf primordium is an upward bulge at one side of the stem apex, at the tip of which is a distinct wedge-shaped cell (figs. 3, 15). As the leaf develops a series of meristematic cells extends from the leaf apex back in two lateral rows along the leaf margin. The broadening of the leaf is brought about by the marginal series of cells cutting off segments alternately from the upper and lower side (fig. 14). The young leaf gives rise to multicellular glandular hairs (fig. 14).

While the petioles of *Camptosorus* are green and small air spaces are common in the cortex, there are no stomata in the epidermis except on the abaxial surface of the lateral wings. In the wings the intercellular spaces are larger and communicate directly with those of the cortex of the petiole proper. The stomata on the abaxial faces open into the intercellular spaces of the wing.

The mesophyll of the mature leaf, which regularly is seven cells thick, is composed entirely of spongy parenchyma (fig. 16). The spongy cells in the lower half may be slightly larger and more irregular than those in the upper half; they may also have fewer chloroplasts. The upper epidermal cells are more or less regular, contain a few chloroplasts, and are covered with a layer of cutin; the lower epidermal cells, which are more irregular, also contain a few chloroplasts but have less cutin.

Stomata are found only in the lower epidermis, the long dimension of each parallel to the length of the leaf. The guard cells have many chloroplasts. Each



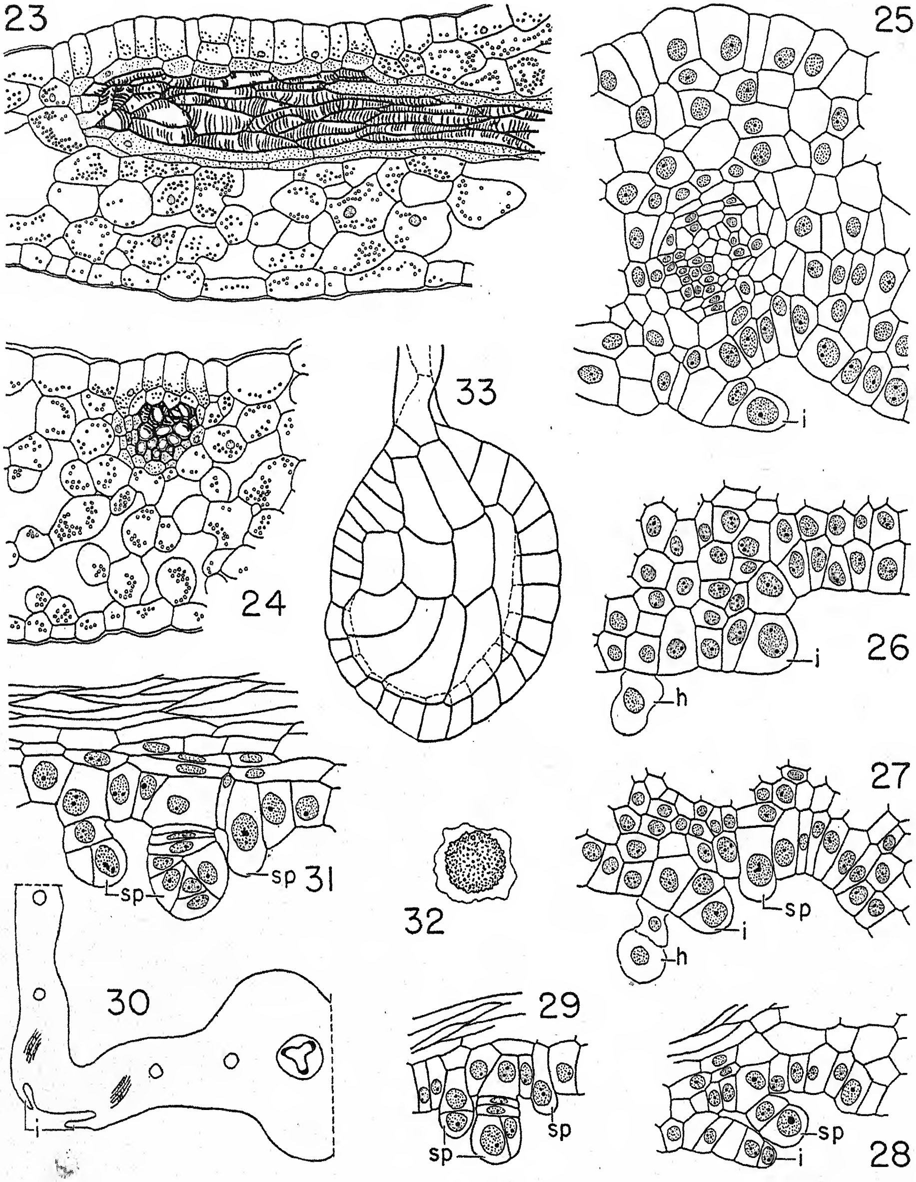
FIGS. 8–22.—Figs. 8–13, cross sections of petiole from base to leaf showing origin of first lateral veins. Fig. 14, cross section of young leaf showing marginal meristematic cells and many hairs. Fig. 15, detail of longisection of leaf primordium showing apical cell. Fig. 16, cross section of mature leaf near midrib showing spongy character of mesophyll and stomata in lower epidermis. Figs. 17–21, surface views of lower epidermis of young leaves showing development of guard cells. Fig. 22, detail of lateral vein in leaf: *chlor*, chloroplast; *meso*, mesophyll cell; *x*, xylem.

pair of guard cells is almost surrounded, except for one end, by a single epidermal cell. Development of the stomatal apparatus is similar to that described and illustrated by ATKINSON (1) and SADEBECK (40) for many ferns. In an epidermal cell of a young leaf a corner is cut off; this is followed by differential growth of the two cells, the outer growing faster than the guard cell initial. The latter grows more on the side toward the epidermal cell, so that the initial becomes surrounded. This initial divides in half, parallel to the long axis of the leaf, forming the guard cells (figs. 17–21). A cross section of a pair of such cells shows moderate guard cell thickening (fig. 16). The cutin on the lower epidermis is not so heavy as on the upper but is heavier on the guard cells, where it forms a little ridge seen in cross section on the outer face of the cells around the stoma. Around the margin of the leaf the cell walls of epidermal and mesophyll cells thicken; there is less intercellular space, and the cutin becomes much heavier.

The structure of the midrib is like that of the petiole which has been described. The small lateral veins diverging from the midrib are almost terete and are surrounded by a complete endodermis (fig. 22). A pericycle, not always continuous, is present. The xylem is largely spirally thickened, but in the larger veins tracheids with scalariform thickenings are found. A small amount of phloem is present on the abaxial side of the bundle. The lateral veins are located near the middle of the tissues of the leaf, but as the vein approaches the leaf margin it rises to the surface and the number of tracheids increases (figs. 23, 24). Cells of the upper epidermis immediately above this enlarged tip of the vein are thin-walled and not covered with cutin, as is the rest of the leaf surface. This simple type of hydathode is formed on the end of every lateral vein, even in the acuminate tip of the leaf. The hydathodes are oval in outline, and the length varies from one and a half to three times the width. As seen in section (figs. 23, 24), the margins of the hydathodes may be slightly depressed, but the central portion is as high as or higher than the surrounding leaf surface. Attempts to induce guttation were not successful.

SORUS.—The sori are irregularly placed on the reticulated veins. Occasionally there are sori back to back on one vein, but in general, if they are paired at all, they are seated on separate veins and face one another (fig. 1). On the lateral veins, if they occur singly, they may either face toward or away from the midrib. They are often found on the veins running parallel to the midrib, with the indusium opening toward the midrib, particularly near the tip of the leaf, where they may reach a length of 5 mm. (fig. 1*d*). When young, the sorus is covered by a membranous indusium which opens along the side opposite the vein on which the sorus is seated. As the sorus matures the indusium pulls back, and the long-stalked sporangia protrude from beneath it.

The sorus makes its appearance on the under surface while the leaf is still very small but after the vascular tissue of the main vein has begun to mature and the lateral veins to differentiate. The first change indicating development of the sorus is evident directly below a vein, where the indusium forms a ridge along the margin of a slight depression on the lower surface of the leaf (fig. 26). The cells of the ridge divide, producing a definite flap (figs. 25, 27, 28, 30). The indusium remains one cell in thickness



FIGS. 23–33.—Fig. 23, longisection of hydathode. Fig. 24, cross section of hydathode. Figs. 25–29, 31, development of sorus: *i*, developing indusium; *sp*, developing sporangium; *h*, hair. Fig. 30, cross section of young leaf showing two developing sori facing each other. Fig. 32, mature spore. Fig. 33, full-sized but still immature sporangium.

through most of its expanse but may become two or three cells thick at the base. Young indusial flaps, opening toward one another and each seated on a separate vein, are frequently seen (fig. 30).

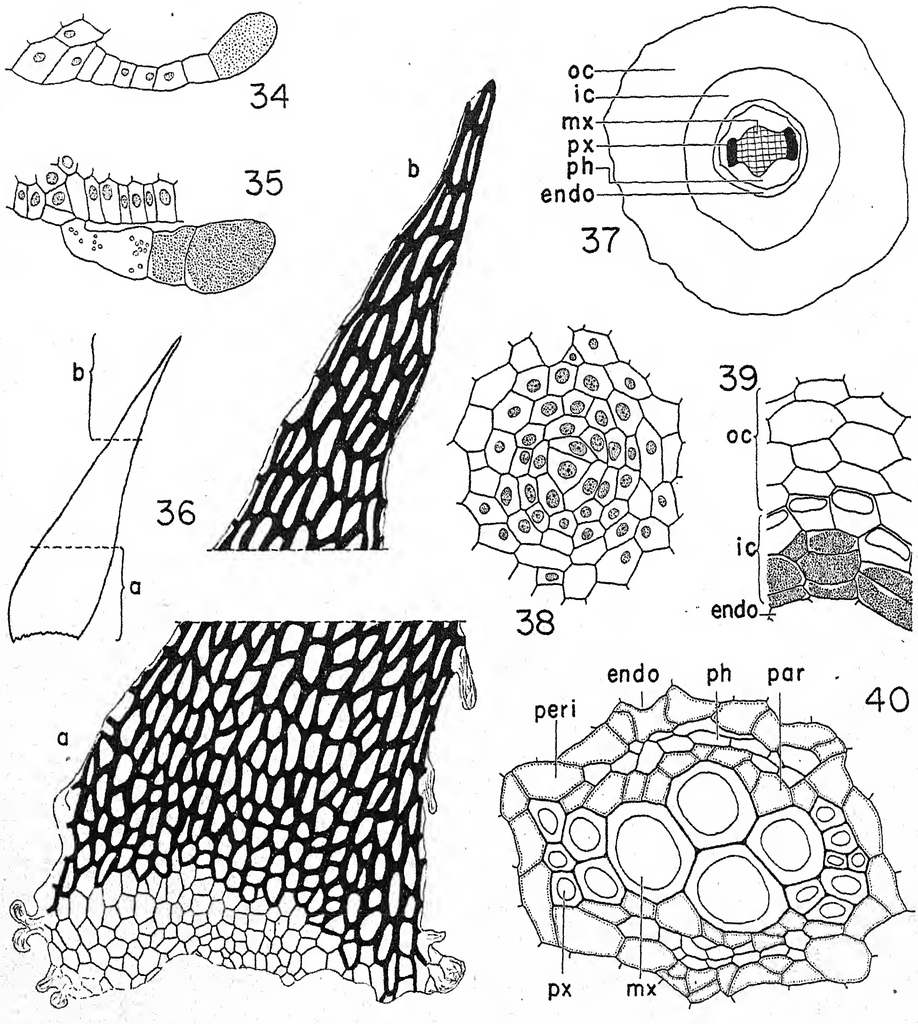
While the indusium is still very small certain epidermal cells at its base become conspicuous owing to the enlarged nuclei (fig. 25). At first there is a suggestion of basipetal sequence in the appearance of these sporangial initials, but later they are definitely mixed. Instead of forming on a well-developed receptacle, the initials arise from cells directly under a vein which is near the lower surface of the leaf in the region of the sorus (figs. 25, 29, 30).

The epidermal cell from which the sporangium will arise possesses a large nucleus, stains more readily, and soon becomes papillate (figs. 27, 29, 31). The first division of this projecting cell is parallel to the surface of the leaf, forming a stalk cell which protrudes slightly beyond the epidermis, and a terminal cell (fig. 29). The stalk cell may undergo further divisions parallel to the leaf surface, but the cells thus formed do not lengthen until later (figs. 28, 29, 31). Then by successive oblique divisions in the end cell a tetrahedral cell is formed at the tip (figs. 29, 31). The outer portion of this is cut off, leaving an inner sporogenous initial surrounded by a layer of sterile cells (fig. 31). Subsequent development follows the usual pattern, and the mature sporangium has an annulus that is vertical and interrupted (fig. 33). The cell next to the stalk is not indurated. The number of indurated cells is variable; in different sporangia sixteen to twenty-seven were counted, with an average of twenty in twenty-three sporangia. There are four stomium cells which are broadened laterally. In the development of the young sporangium the series of cross walls in the stalk cell produces a one-rowed stalk rather than the three-rowed type which ordinarily develops by a series of oblique divisions. In the mature sporangium, the greater length of the stalk consists of a single row of cells which passes over into two or three where it joins the capsule. The mature spores are bilateral, with a dark brown, slightly rough coat and a perispore (fig. 32).

Dermal appendages.—Both hairs and scales are found on *C. rhizophyllus*. Multicellular hairs with a large glandular cell at the tip are common on both surfaces of the young leaves (fig. 35) but disappear from the upper surface as the leaf matures, although a few may persist on the lower surface, where they appear as dark brown spots. The rhizome tip and petiole bases are covered by glandular hairs (fig. 34) and by scales about 1 mm. in length and 0.3 mm. in width at the base. The scales (fig. 36) are narrow and acuminate, with thin outer walls and with the lateral walls heavily thickened. They are one cell in thickness from base to tip and are attached by several cells at the base. In most cases the tip ends in a large glandular cell similar to that of the hairs on the leaf.

Root.—A single root is associated with a leaf base. It diverges endogenously from a meristele, usually below—but occasionally above—the opening of the leaf gap (fig. 4). The root primordium can be seen arising close to the primordium of the leaf with which it is to be associated. Protoxylem, chiefly small scalariform tracheids, does not appear until the root has progressed far enough through the cortex to break the epidermis. At the tip of the root is a single pyramidal apical cell (fig. 38). Divisions of this cell in three planes give rise to cells the derivatives of which form the

vascular, cortical and epidermal tissues of the root, while divisions on the fourth side give rise to the root cap.



FIGS. 34–40.—Fig. 34, glandular hair from rhizome and petiole base. Fig. 35, glandular hair from leaf. Fig. 36, diagram and partial detail of scale from rhizome and petiole base; glandular tip not shown. Fig. 37, cross section of root: *oc*, outer cortex; *ic*, inner cortex. Fig. 38, detail of cross section of root apex showing apical cell. Fig. 39, detail of cortex of root. Fig. 40, detail of stele of root.

pass outward, either directly or obliquely. In a few instances roots have been observed to branch immediately after they

The root may remain within the leaf base with which it is associated for a considerable distance before it becomes free and turns downward, or it may bend downward in the cortex almost immediately after it is given off and then become free from the rhizome. The roots may be 0.5 mm. in diameter but are often less.

Several layers of cortical cells of the root near the endodermis are more or less thickened on their radial and inner

tangential walls (figs. 37, 39). A continuous endodermis separates the stele from the cortex (figs. 37, 40). The pericycle is continuous and usually only one row of cells in thickness. The xylem is diarch; the two protoxylem strands are in contact with the pericycle. A small amount of phloem is present on either side of the xylem and separated from it by a few parenchyma cells.

Discussion

The two species of *Camptosorus*, when not looked upon as forming a distinct genus, have often been placed in one of the genera now included in the genus *Phyllitis*. *P. scolopendrium*, the best known species of the genus, is larger than *Camptosorus* and has simple leaves growing in spiral succession from an upright stock. The scales found on the rhizome tip and leaf bases have a glandular cell at the tip (3), as do those found in *C. rhizophyllus*. In some species of *Phyllitis*, as *P. scolopendrium*, the venation is free, but in others, such as the Mexican species *P. nigripes* (Fée) O. Ktze, the venation is reticulate (9, 7). The rhizome of *P. scolopendrium* contains a radial dictyostele with a single root trace given off below the opening of a leaf gap. The leaf gap opens above the origin of the root trace and from each margin a strap-shaped vascular strand is given off (3, 22, 23). In the leaf stalk the strands may fuse, forming an X-shaped trace in which the upper arms of the figure are stronger (34, 35, 36, 43). The origin of the lateral veins is from the adaxial tips of the trace. The petiole of *P. scolopendrium* has small lateral wings for a short distance below the blade and stomatal strips for a longer distance (32). The leaf blade is similar to that of *C. rhizophyllus* in having chloroplasts in the epidermal cells and no palisade tissue; the mesophyll is nine cells thick (19, 43, 34, 35). A close resemblance to *C. rhizophyllus* is found in the soral arrangement and development and in the sporangium and spores of *P. scolopendrium*. The soral arrangement of *C. rhizophyllus* is less regular than that of *P. scolopendrium* but is strikingly like that of *P. nigripes*, which has reticulated veins (7, fig. 672). Bower (3, 4) describes the origin of the sorus on the young leaf of *Phyllitis:* the indusium arises at the edge of a slight depression, and the sporangia arise at the base of the depression in a sequence at first slightly basipetal (3). The mature sporangium of *Phyllitis*, described by Strasburger (43), has an incomplete vertical annulus with a varying number of indurated cells and a stalk which passes over from a single to a double row of cells. The spore is bilateral and has a rough coat and perispore (21).

The resemblance between *Camptosorus* and *Phyllitis* consists in the habit of the two ferns, in the scales with a glandular tip, in the unperforated dictyostele with binary leaf traces and single root associated with a leaf base, in the geminate arrangement of sori, in the development of the sorus at first basipetal and then mixed, in the early appearance of the indusium, in the sporangium with stalk one-rowed at the base, and in the bilateral spore with perispore. As the relationship between *Camptosorus* and *Phyllitis* seems highly probable, the two genera will be discussed together.

The two groups to which they would naturally be compared are the Aspleniods and the Blechnoids because of the elongated, superficial sori. The relationship of these groups to each other is not established. Bower (4), who classes *Camptosorus* and *Phyllitis* with the Blechnoids, considers that similarities

between this group and the Asplenioids afford a good example of convergent evolution. On the other hand, CHRISTENSEN (9) places the two genera in the Asplenioids, which group he places between the Dryopteroids and the Blechnoids.

In habit the Blechnoids vary from small forms to some that are subarborescent. The smaller forms, which may have simply pinnatifid leaves, show similarity to *Camptosorus* and *Phyllitis* in habit and anatomy. The dermal appendages are broad scales and hairs with a large terminal glandular cell. The vascular system is dictyostelic, the number of overlapping leaf gaps depending on the size of the fern. In *B. patersoni*, one of the smaller forms, a single root trace passes off just below the base of the leaf gap, and above this from right and left of the gap two equal leaf traces arise (3); this is similar to that described for *C. rhizophyllus*. DAVIE (15–17) has found that many species of *Blechnum*, including *B. patersoni* and *B. punctulatum* var. *krebsii*, have an "extra-marginal" type of pinna-trace. In this type of trace the portion which comes from the adaxial side of the leaf trace is nipped off from the back of the hook, the adaxial face of the curved leaf trace; the extreme tip of the adaxial portion is continued upward as part of the leaf trace. This differs from *Camptosorus* and *Phyllitis*, which give rise to the first lateral veins in a "marginal" manner.

BOWER bases his classification of *Camptosorus* and *Phyllitis* among the Blechnoids on the interpretation of the phyletic development of the sorus, with emphasis on the intermediate conditions between the Blechnoid type of sorus and that of *Camptosorus* and *Phyllitis* found in *B. punctulatum* var. *krebsii*. HOOKER (24) was the first to suggest the similarity of this species of *Blechnum* to *Phyllitis*. *Camptosorus* offers the most interesting comparison, as its sori vary from parallel ones of the Blechnoid type in the excurrent apex of the leaf to soral arches in the middle region and irregular arrangement (single, paired face to face, paired back to back) in the basal portions —conditions very nearly matched in *B. punctulatum* var. *krebsii* (4). The sorus of *Blechnum* is like that of *C. rhizophyllus* in showing a slightly gradate sequence at first but differs from it in the formation of the indusium after the sporangia have appeared. The sporangia of the species of *Blechnum* studied by SCHNARF (41) differ from those of *C. rhizophyllus* in having a three-rowed stalk, but the spores are bilateral and have a perispore (21) as in the latter species.

The genus of the Asplenioids which *C. rhizophyllus* most closely resembles in habit is *Asplenium*. Many of the latter genus are rock-loving ferns with simple to decompound leaves. They often propagate vegetatively, as does *Camptosorus*. Mature guard cells in the leaves of *Camptosorus* and species of *Asplenium* are alike (11). The lattice-thickening of the scales on the rhizome of species of *Asplenium* is like that of the scales of *C. rhizophyllus*. The rhizomes of such species as *A. alatum*, *A. obtusatum*, and *A. marginatum* show construction similar to that of the walking fern. The axis contains a dictyostele; the two straps of the leaf trace arise from either side of the leaf gap and the root traces from the meristele below the opening of the leaf gap. The leaf trace bundles may fuse upward to form a vascular tract, X-shaped in cross section (3, 4, 34, 35). DAVIE (15–17) has found that species of *Asplenium* (*A. bulbiferum*, *A. obtusatum*, *A. ruta-muraria*, *A. trichomanes*, *A. pinnatifidum*) have a "marginal" type of

pinna-trace. In the marginal type the first indication of the preparation for departure of the pinna-trace, as the leaf trace is followed up the petiole, is an extension of the mass of tracheids on the adaxial face of the petiole, that portion of the trace nearest the pinna. This tip is cut off to supply the pinna. This is similar to *Camptosorus* and *Phyllitis*, which, although not pinnate, give off the first lateral veins in a marginal manner.

Typically the sorus of *Asplenium* arises on the fertile vein, and the indusium opens so that it faces the midrib of the fertile frond, pinna, or pinnule. Development of the sorus is like that of *C. rhizophyllus* in the appearance of the indusium before the sporangia, but differs in that the sporangia arise in a distinctly mixed sequence, with no suggestion of basipetal arrangement (3). Sporangia, illustrated by SCHNARF (41) and MÜLLER (33), have a long, one-rowed stalk similar to that found in *C. rhizophyllus*. The spores are bilateral, with a perispore (21).

C. rhizophyllus and *Phyllitis* show close resemblance to both *Asplenium* and small forms of *Blechnum* in habit, in the presence of scales on the rhizome, in the unperforated dictyostele with binary leaf traces, in the single root associated with a leaf base, and in the bilateral spore with perispore. The arrangement of the sori on the mature leaf of *C. rhizophyllus* and of *Phyllitis* is like that of *B. punctulatum* var. *krebsii*. A glandular cell is found at the tip of the scales in *C. rhizophyllus*, in *Phyllitis*, and in *Blechnum*. Similarity between *C. rhizophyllus* and *Asplenium* is found in the lattice-thickened scales, in the crescent-shaped leaf trace bundles fusing in the petiole or midrib of the leaf, in the marginal type of pinna-trace, in vegetative reproduction, and in the sporangium with a one-rowed stalk. Relationship is also suggested by the possibility of hybridization between the two genera (2, 6, 10, 12, 13, 14, 26, 31, 42, 44). Although there is more evidence for allying *Camptosorus* with the Asplenioids than with the Blechnoids, difficulty is found in the interpretation of the phyletic development of the soral arrangement. There is no transition form between *Asplenium* and *Camptosorus*, such as *Blechnum punctulatum* var. *krebsii* provides between *Blechnum* and *Camptosorus*. However, more will need to be known about both species of *Camptosorus*, about *Phyllitis* (particularly those species with reticulate venation), and about various species of both *Asplenium* and *Blechnum* before the relation of the Asplenioids and Blechnoids to each other and the systematic position of *Camptosorus* and *Phyllitis* can be determined with any degree of certainty.

Summary

1. The rhizome of *Camptosorus rhizophyllus* is radial, with leaf bases diverging from all sides. It has an unperforated dictyostele with curving meristeles bordering the leaf gaps. In transverse section ordinarily three meristeles are found.

2. The two parts of the binary leaf trace arise individually in the lower half of the leaf gap, one from each side of it. They merge in the petiole, forming a trace V-shaped in cross section, or occasionally with a suggestion of an X-shape.

3. The lateral veins arise from the margin of the adaxial arms of the leaf trace or midrib, a marginal type of pinna-trace.

4. A single root is associated with a leaf base. The root trace diverges from a meristele just before it divides to form

the leaf gap of the leaf with which the root is associated.

5. Dermal appendages are of two types—multicellular glandular hairs on young leaves, with a few persisting on the lower surface of mature leaves; and lattice-thickened scales, often with a glandular tip, on the petiole base and the rhizome tip.

6. Leaves are covered with a heavy coat of cutin; the epidermal cells contain chloroplasts; and stomata are present in the lower epidermis; the mesophyll is not differentiated into spongy and palisade tissue. Simple hydathodes are found on the upper surface of the leaf near the margin at the tips of the lateral veins.

7. The sori are oblong to linear and are scattered irregularly on the reticulated veins, some borne singly and facing in different directions and some in pairs facing each other.

8. Each sorus is seated on a vein and covered by a membranous indusium opening along the side of the sorus away from the vein on which it is seated.

9. The sorus develops when the leaf is still young but after the midrib has begun to mature and the lateral veins to differentiate. The indusium appears first and is followed by development of the sporangia.

10. The sporangia appear in a sequence at first slightly gradate but later distinctly mixed.

11. The sporangia are variable in size, and the annulus contains a variable number of indurated cells, with an average of twenty. The stalk is one-rowed, passing over to two or three rows at the base of the capsule.

12. *Camptosorus* shows resemblance to both *Asplenium* and small forms of *Blechnum* in habit, dermal appendages, and rhizome anatomy; to *Asplenium* in leaf trace, pinna-trace, vegetative propagation, sporangia, and spores; and to *Blechnum punctulatum* var. *krebsii* in soral arrangement. There appears to be more evidence for allying *Camptosorus* (and what seems to be its closest relative, *Phyllitis*) to the Asplenioids than to the Blechnoids.

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LITERATURE CITED

1. Atkinson, G. F., The biology of ferns. New York. 1894.
2. Benedict, R. C., Do ferns hybridize? Science n.s. **33**:254–55. 1911.
3. Bower, F. O., Studies in the phylogeny of the Filicales. IV. *Blechnum* and allied genera. Ann. Bot. **28**:363–432. 1914.
4. ———, The ferns. Vol. III. Leptosporangiate ferns. Cambridge. 1928.
5. ———, Primitive land plants. London. 1935.
6. Braun, E. Lucy, A new fern hybrid—*Asplenium cryptolepis* × *Camptosorus rhizophyllus*. Amer. Fern Jour. **29**:133–135. 1939.
7. Christ, H., Die Farnkräuter der Erde. Jena. 1897.
8. Christensen, Carl, Index Filicum. Hafniae. 1906.
9. ———, Chap. XX: Filicinae (pp. 522–550). Manual of pteridology. Ed. by Franz Verdoorn. The Hague. 1938.
10. Copeland, E. B., Two fern monstrosities. Bot. Gaz. **34**:142–144. 1902.
11. ———, The mechanism of stomata. Ann. Bot. **16**:327–364. 1902.
12. Coulter, J. M., and Coulter, M. S., Editorial. Bot. Gaz. **7**:37. 1882.
13. Davenport, G. E., § 212. *Asplenium ebenoides* R. R. Scott. Bull. Torr. Bot. Club **6**:200. 1878.
14. ———, Two new ferns from New England. With some observations on hybridity and nomenclature. Bot. Gaz. **19**:492–497. 1894.
15. Davie, R. C., The pinna-trace in the ferns. Trans. Roy. Soc. Edinburgh **50**:349–387. 1914.
16. ———, On the leaf-trace in some pinnate leaves.

Trans. Roy. Soc. Edinburgh 52:1–36. 1917–1918.

17. ———, A comparative list of fern pinna-traces, with some notes on the leaf-trace in the ferns. Ann. Bot. 32:233–245. 1918.

18. DIELS, L., Polypodiaceae. In ENGLER and PRANTL's Die natürlichen Pflanzenfamilien. Leipzig. 1902.

19. GLOSS, MARY ELGIN, Mesophyl of ferns. Bull. Torr. Bot. Club 24:432–435. 1897.

20. GOEBEL, K., Ueber Regeneration im Pflanzenreich. Biol. Central. 22:385–397. 1902.

21. HANNIG, E., Über das Vorkommen von Perisporien bei den Filicinen nebst Bemerkungen über die systematische Bedeutung derselben. Flora 103:321–346. 1911.

22. HAYATA, BUNZO, On the systematic importance of the stelar system in the Filicales. I. Bot. Mag. Tokyo 41:697–718. 1927. (In Japanese.)

23. ———, Über die systematische Bedeutung des des stelären Systemes in den Polypodiaceen. Flora 124:38–62. 1929–1930.

24. HOOKER, W. J., Species Filicum. Vol. III. London. 1860.

25. HOOKER, W. J., and BAKER, J. G., Synopsis Filicum; or a synopsis of all known ferns, including the Osmundaceae, Schizaeaceae, Marattiaceae, and Ophioglossaceae. 2d ed. London. 1874.

26. HOYT, W. D., Physiological aspects of fertilization and hybridization in ferns. BOT. GAZ. 49: 340–370. 1910.

27. KUPPER, WALTER, Über Knospenbildung an Farnblättern. Flora 96:337–408. 1906.

28. LINNAEUS, C., Species Plantarum. Vol. II. Holmiae. 1753. Facsimile edition. Berlin. 1907.

29. MCVEIGH, ILDA, Vegetative reproduction in *Camptosorus rhizophyllus*. BOT. GAZ. 95:503–510. 1934.

30. ———, Apical growth of the leaves of *Camptosorus rhizophyllus*. Amer. Jour. Bot. 23:669–673. 1936.

31. MAXON, W. R., Notes on the validity of *Asplenium ebenoides* as a species. BOT. GAZ. 30: 410–415. 1900.

32. MITCHELL, MARGARET R., Note on the lateral lines of the petioles of ferns. Trans. Proc. Bot. Soc. Edinburgh 29:105–112. 1924.

33. MÜLLER, CARL, Zur Kenntniss der Entwicklungsgeschichte des Polypodiaceensporangiums. Ber. deutsch. Bot. Ges. 11:54–72. 1893.

34. PARMENTIER, M. P., Recherches sur la structure de la feuille des fougères et sur leur classification. Ann. Sci. Nat. Bot. 8 sér. 9:289–361. 1899.

35. PELOURDE, FERNAND, Recherches anatomiques sur la classification des fougères de France. Ann. Sci. Nat. Bot. 9 sér. 4:281–372. 1906.

36. ———, Recherches comparatives sur la structure des fougères fossiles et vivantes. Ann. Sci. Nat. Bot. 9 sér. 10:115–147. 1909.

37. PICKETT, F. L., Resistance of the prothallia of *Camptosorus rhizophyllus* to desiccation. Bull. Torr. Bot. Club 40:641–645. 1913.

38. ———, The development of the prothallium of *Camptosorus rhizophyllus*. BOT. GAZ. 57:228–238. 1914.

39. ———, Some ecological adaptations of certain fern prothallia—*Camptosorus rhizophyllus* Link, *Asplenium platyneuron* Oakes. Amer. Jour. Bot. 1:477–498. 1914.

40. SADEBECK, R., Pteridophyta. Vol. I, ENGLER and PRANTL, Die natürlichen Pflanzenfamilien. Leipzig. 1902.

41. SCHNARF, KARL, Beiträge zur Kenntnis des Sporangienwandbaues der Polypodiaceae und der Cyatheaceae und seiner systematischen Bedeutung. Sitz. Kais. Akad. wiss. mathnaturw. Klasse. 113–114:549–573. 1904–1905.

42. SLOSSON, MARGARET, The origin of *Asplenium ebenoides*. Bull. Torr. Bot. Club 29:487–495. 1902.

43. STRASBURGER, E., Handbook of practical botany. Ed. from German by W. HILLHOUSE. London. 1887.

44. UNDERWOOD, L. M., The habitats of the rarer ferns of Alabama. BOT. GAZ. 22:407–412. 1896.

45. WHERRY, E. T., A chemical study of the habitat of the walking fern, *Camptosorus rhizophyllus* (L.) Link. Jour. Wash. Acad. Sci. 6:672–679. 1916.

46. ———, Observations on the habitat of certain ferns. Amer. Fern Jour. 7:110–112. 1917.

47. WUIST, ELIZABETH D., Branched prothallia in the Polypodiaceae. Bull. Torr. Bot. Club 43: 365–383. 1916.

48. YARBROUGH, J. A., The foliar embryos of *Camptosorus rhizophyllus*. Amer. Jour. Bot. 23:176–181. 1936.

SPORULATION IN SACCHAROMYCES CEREVISIAE[1]

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Introduction

Sporulation in yeasts was probably first studied by DE SEYNES (19), who reported that round spores found "in the surface of the water" germinated to produce elongate cells in a mixture of wine and water, but that these elongate cells produced spores again when transferred to a more dilute medium. REES (16) found that when yeast was planted on the cut surface of various vegetables (cooked or raw), growth continued until the fourth day, when budding stopped. On the fifth day the vacuoles in the cell disappeared and the protoplasm became coarsely granular. Spore formation occurred regularly on the sixth day. Spores also appeared on the sixth day if fresh yeast from beer vats or wine must was transferred to the cut surface of carrot or potato. REES did not find spores in old lagering vats or in compressed yeast, but washed yeast from these sources placed in a beaker in a layer approximately 4 mm. thick and protected from dust sporulated abundantly in about 3 weeks.

ENGEL (4) devised the classic method of inducing yeasts to sporulate by transferring the cells from a nutrient broth to plaster of Paris blocks. HANSEN (7) used this method successfully. GRAHAM and HASTINGS (6) have improved the technique considerably by making the gypsum slants in test tubes, thus reducing the danger of contamination. BEYERINCK (2, 3) obtained yeast spores by transferring the suspensions to slants made from agar that had been fermented and subsequently washed. He preferred this method to use of the gypsum block, probably because less contamination was encountered.

HANSEN and BEYERINCK considered it essential that the yeast cells be well nourished before transferring them to the aerating surface, but they did not specify any nutrients aside from beer wort or grape must. They believed that sporulation occurred when the "well-nourished" cells were deprived of all nutrients, and HANSEN'S blocks and BEYERINCK'S purified agar were flooded with distilled water. They agreed that starvation conditions and an abundance of oxygen were essential.

GORODKOWA (5) developed an agar containing 1% peptone and 0.25% glucose. On this medium sporulation occurred after 3–4 days. She showed that on a similar medium containing 5% glucose no spores were formed, indicating that the "starvation" conditions essential to sporulation involved primarily the sugars in the substrate rather than other components.

WELTEN (20) challenged the view that starvation was essential to sporulation. He found that on prune extract agar, yeasts sporulated well. He even doubted the necessity for oxygen, since in his experiments, colonies imbedded in the agar sporulated as well as those on the surface. He found that yeasts grown in pear extract or beer wort did not sporulate so well as those grown on prune extract agar. But sporulation occurred when the washed yeast grown on prune extract was placed on glass plates, filter paper, or sterile washed agar slopes, if a drop of prune extract were added. If no prune extract were added, no spores were produced. GORODKOWA'S and WELTEN'S work proved that starvation alone is not the complete explanation of the phenomenon. WELTEN showed further that acidity of the medium in which sporulation occurred was important, no

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spores occurring in an alkaline milieu. WELTEN also found that more spores were produced in concentrated than in dilute prune extract, also that a small amount of $MgSO_4$ aided sporulation. Young cells were not essential; those 3–4 days old sporulated better than those 1–2 days old.

BALTATU (1) found that spores were produced by mycodermas in grape juice but usually germinated immediately, so that they could be detected only by periodic examination of the culture. The addition of very small amounts of acetic acid and grape juice to the water in which the gypsum blocks were placed increased sporulation.

MRAK (13) discovered that many yeasts sporulated well on the slopes of an agar medium containing a mixture of vegetable (cucumber, beet, potato, and carrot) extracts. This is clearly a medium containing only a small amount of sugar, which is probably soon exhausted to about the concentration of GORODKOWA's.

NICKERSON and THIMANN (14) found that conjugation and sporulation occurred more abundantly in a *Zygosaccharomyces* when many dead cells were present and postulated some stimulating substance derived from the dead cells. MANEVAL (12) found that sporulation occurred in *Saccharomyces* on the outside of compressed yeast cakes, but he did not comment on the presence of dead cells. NICKERSON and THIMANN also discovered that an extract from *Aspergillus* increased conjugation and sporulation, and later (15) they showed that riboflavin and sodium glutarate were probably the substances in the extract responsible for the stimulation.

The general conclusions from these data are that a specific pre-sporulation nutrient is essential to abundant sporulation. If this nutrient is satisfactory and the sugar content of the medium low enough, sporulation may occur even on the agar slant, as in GORODKOWA's or MRAK's media. Generally, the yeast should be removed from the substrate, especially if its sugar content is high, and placed on gypsum. The water saturating the gypsum slant should be acid and should contain by-products of yeast growth and possibly some substances produced by the death and disintegration of yeast cells.

Genetical analysis requires an abundance of large viable 4-spored asci, and the present paper reports an investigation of the conditions controlling sporulation in *Saccharomyces cerevisiae*. The objective was to develop an optimal medium for the production of spores, and especially to reduce the time required to obtain them. Grape juice was found a satisfactory pre-sporulation nutrient for some yeasts but was ineffective with others. GORODKOWA's medium and carrot agar were ineffective generally. Yeasts vary extremely in their capacity for sporulation, and it was essential to develop a medium that would activate even the poorest sporulating cultures. The study of pre-sporulation nutrition was extended and an extremely effective combination of nutrients developed. There seems to be a general correlation between the size of the ascospores and their vigor, and media which produce the larger spores were favored. Since the larger asci are easier to dissect, this characteristic has a twofold value. Immature spores are usually non-acidfast, and components of the media which reduce the number of such spores to a minimum have been selected. Throughout this work counts have been made or percentages of sporulation estimated on the slides after staining, but different

numbers of strains as well as different strains were tested on the various media and changes in technique developed as the work progressed, so that comparisons are confined to each particular batch. For example, the designation ++++, in the earlier tests on grape-apricot-apple mixtures, does not have the same significance as it has in the later tests. In the earlier tests 4-spored asci appeared only rarely, and presence or absence was a sufficient means of distinction. As the media improved, the numbers of 4-spored asci increased, until finally media were judged on their ability to produce large 4-spored asci and on their comparative percentages. In any event, the percentage always played an important part in the selection of media. Sporulation was more abundant directly on the slants of simple potato agar than on most of the media tested, but this medium was not used because the asci were small and predominantly 2-spored, even when cultures genetically capable of producing 4-spored asci were used. Cells grown on a substrate containing sugar became much larger than those grown on a vegetable slant. Sporulation does not occur on a rich sugar medium, and therefore transfer to gypsum is required. However, since this extra step results in the production of larger spores, the advantage more than counterbalances the extra work.

Investigation

Grape-apricot-apple mixtures

Since Baltatu showed that grape juice was an effective pre-sporulation nutrient, it seemed probable that other fruits on which yeast occurs naturally might also be effective. The most notable of these is the apricot.

The effect on sporulation of various combinations of (*a*) bottled grape juice, (*b*) canned apricot juice, and (*c*) commercial bottled apple cider was tested. The fluids were each diluted with equal quantities of water and made into agar medium by adding 3% of agar and neutralizing with 1% of powdered $CaCO_3$. The combinations were made by mixing equal parts of the agars. Slants of the media were prepared, without filtering, and inoculated with yeast. After 2 days they were transferred to gypsum slants, and several days later slides were prepared and the number of spores estimated. The average production of spores for three strains of yeast is as indicated:

Grape	+
Apricot	++
Apple	+
Apricot-apple	+
Grape-apple	+
Grape-apple-apricot	+
Grape-apricot	++++*

* 4-spored asci present.

Commercial apple cider apparently suppresses sporulation, and a mixture of grape and apricot is much more effective than either alone. The cells from the grape-apricot mixture produced the only 4-spored asci found.

Tomato-prune-potato mixtures

The effect on sporulation of combining the grape-apricot medium with (*a*) canned tomato juice, (*b*) juice from canned prunes, (*c*) juice from canned figs, and (*d*) juice from canned potatoes was tested. The spores produced on the simple tomato medium were obviously aborted. They were tiny and were suspended in a blue-stained epiplasm. None of these combinations was as good as the grape-apricot mixture, with the possible exception of potato. Malt extract was also found to be ineffective by itself or in combination.

Vegetable-fruit mixtures

Tests using Mrak's medium containing carrot, cucumber, beet, and potato showed it to be effective in inducing some strains to sporulate but relatively ineffective with others. Media were made combining cucumber, beet (roots and stems), and potato. About 200 gm. of each were ground in a meat grinder, boiled, and squeezed out through cheesecloth. Equal portions of each of these extracts and grape-apricot juice were used in the agar. Carrot was not used, since it had not been found useful in our earlier work. Mrak obtained spores directly on the agar slants without transfer to gypsum. The following list shows the average sporulation which occurred on the slants of these media after 7 days with five different yeast strains:

Grape-apricot	+
Grape-apricot-cucumber	+
Grape-apricot-beet	+
Grape-apricot-potato	+
Grape-apricot-beet-potato	++
Grape-apricot-cucumber-potato	++
Grape-apricot-cucumber-beet	++
Grape-apricot-cucumber-beet-potato	++++*
Beet	+++*
Potato	++++
Cucumber-beet	++
Cucumber-potato	+
Beet-potato	+
Cucumber-beet-potato	+

* 4-spored asci present.

The media containing grape-apricot juices produced fewer spores on the slants than the vegetable media alone or in combination. The fruit media are relatively sweet and the cells on these media are large, with a large central vacuole. This agrees with Gorodkowa's findings that a decrease in sugar is essential to spore formation directly on the slant. The cells on the vegetable media are medium-sized and densely staining. The spores on the vegetable media were all acidfast, but many of them on the slants containing grape-apricot juice were nonacidfast. The most abundant production of spores occurred on potato agar, but the asci were almost exclusively 2-spored. Cucumber-beet-potato medium is the poorest. The beet extract is the most potent producer of 4-spored asci. No culture failed to produce 4-spored asci on beet agar.

As the cultures age the number of spores on the vegetable media do not increase materially, but there is a slow increase in the number when the richer grape-apricot mixtures become exhausted. Sporulation of the large vacuolate cells on the grape-apricot combination proceeds rapidly after transfer to a gypsum slant, but such transfer does not materially increase the number of spores on the single vegetable media. Spores are produced on the vegetable media as soon as growth is well started. As more components are added to the grape-apricot mixture, more spores are produced. The 5-component mixture is the best, especially when judged by the number of 4-spored asci. This may be due to the dilution of the sugar by the addition of the vegetable extracts. On the slants containing grape and apricot, spores appear slowly or not at all. The simple grape-apricot combination produces spores only after transfer to gypsum.

Glycerin-beet mixtures

Because the spores on the grape-apricot combinations were often nonacidfast, the effect of glycerin was tested. The vegetable most conspicuously effective in producing 4-spored asci was the beet. A beet agar was made up by using 450 gm. of beet roots and 150 gm. of beet leaves; 1500 cc. of water was added and the mixture heated in the autoclave

and squeezed through cheesecloth. Half of this was made into 3% agar without glycerin and half into 3% agar with 3% glycerin. Slants were inoculated with various yeasts and cells stained after 7 days on the slants, without transfer to gypsum. The addition of glycerin inhibited sporulation on the beet-agar slant, but when the beet-glycerin cultures were transferred to gypsum, sporulation on the gypsum increased markedly. The cells grown on the beet-glycerin agar slants were large, with a big central vacuole similar to that of those grown on the media containing grape-apricot. In both cases the explanation is probably the same—sugar and glycerin increase growth on the slant but inhibit sporulation. Twenty-two strains were tested:

Beet agar	++
Beet-glycerin agar	+
Beet-glycerin agar to gypsum	+++

Cow's milk and egg yolk, both used in culturing acidfast bacteria, proved ineffective in increasing sporulation.

An agar with a base of beet roots and leaves containing 3% glycerin was compared with the grape-apricot-vegetable-glycerin medium. Sixteen strains were tested. Both media were equally effective for the production of 4-spored asci, but the spores from the beet-glycerin agar were larger.

NICKERSON and THIMANN'S observation, and a study of the yeast colony by LINDEGREN and HAMILTON (9), suggested that yeast extract might be an effective agent in ascospore production. The addition of 2% dried brewers' yeast proved extremely effective in increasing both the percentage of sporulation and the number of 4-spored asci. It was also found that a relatively high concentration of grape and apricot juice in the medium produced optimal sporulation, with the proportion of 20% grape juice and 40% canned apricot nectar giving the best results.

FINAL MEDIA AND METHODS

The following pre-sporulation medium was finally chosen:

Beet (leaves) extract	10	cc.
Beet (roots) extract	20	cc.
Apricot juice	35	cc.
Grape juice	16.5	cc.
Yeast (dried)	2	gm.
Glycerin	2.5	cc.
Agar	3	gm.
$CaCO_3$	1	gm.

Water was added to give a final volume of 100 cc. The mixture was steamed for 10 minutes and tubed. Tubes were sterilized at 15 lb. for 20 minutes and slanted. Most strains of yeast will produce spores directly on the slants if allowed to grow for a few weeks. However, if spores are needed sooner, transfer to gypsum is necessary.

ENGEL'S gypsum block method was replaced by the method of GRAHAM and HASTINGS. A mixture of plaster of Paris and water (100 gm. of each) was poured into test tubes and solidified in a slanting position. These slants were dried at 50° C. for 24 hours and autoclaved.

TECHNIQUE.—About 1 cc. of sterilized water is poured over a 3-day growth of yeast on the pre-sporulation medium and allowed to stand 10 minutes, then a thick suspension is made by stirring the yeast cells around in this supernatant water. The yeast suspension is taken up in a pipette and poured over the upper part of the gypsum slant. About 3 cc. of sterile water containing enough acetic acid to bring the pH to 4 is pipetted into the lower half of the gypsum slant. The inoculated gypsum slants are incubated 1–2 days at 25° C.

ACIDFAST SPORE STAIN.—The asci are

smeared on the slide from the gypsum slope. The smear is fixed by heat and placed in a Coplin jar of carbol fuchsin for 20 minutes or longer. Destaining is accomplished by washing rapidly in 30% acetic acid while holding the slide with a forceps. The slide is not rinsed in water before destaining, and this prevents the formation of the precipitate that usually occurs if it is transferred directly to water from carbol fuchsin. Two or three rapid swishes through the acetic acid should immediately be followed by a rinse in water. If the slide is held too long in the acid, or not rinsed quickly enough in water, it will be completely destained. The success of this method depends on critical control of destaining. After the water rinse, methylene blue is used as a counterstain. This procedure stains the spores red and the vegetative cells blue.

Delafield spore stain.—A simpler method is to place a tiny drop of cell suspension on a slide, add a small drop of Delafield's haematoxylin, and cover with a coverglass. This makes a permanent preparation, because the Delafield's contains glycerin and the preparation does not dry out. The cell walls of the vegetative cells and spores are clearly defined against a dark background. Rather good cytological results can be obtained by mixing 7 parts of Delafield's with 3 parts of acetic and using the same procedure. These preparations attain an optimum after about 3 days.

Sporulation in single ascus and single ascospore cultures

Data (8, 11, 17, 18, 21) have already been presented showing that *Saccharomyces cerevisiae* is heterothallic, and that legitimately diploid cultures almost always produce viable 4-spored asci, while single ascospore cultures only rarely do so. The cultures produced by the germination of intact 4-spored asci are usually produced by copulation of genetically different gametes and are therefore heterozygous. The homozygous or haploid cultures produced by single ascospores, when they can be induced to sporulate, produce spores of low viability (fig. 1).

Cultures originating from (*a*) vegetative cells, (*b*) single ascospores dissected from 4-spored asci, (*c*) intact 4-spored asci, and (*d*) 1-spored asci were critically examined in an analysis of four inbred generations of a yeast family, the "D" family (fig. 2). The individual cultures in the pedigree are designated as follows: Arabic numerals indicate asci; capital letters designate the ascospores isolated from asci. For example, D1A is the culture from ascospore A isolated from ascus D1. Four spores were dissected from each 4-spored ascus; in ascus D1 only two spores (D1A and D1B) produced viable colonies. D1C and D1D died and are not listed in the pedigree. Only one spore, D2B, from ascus D2 produced a viable colony. Later investigations showed that single ascospore cultures may be either haploid or illegitimately diploid. However, data concerning the genetic composition of the single ascospore cultures in this pedigree are not available.

Small Roman letters designate subcultures arising from individual colonies obtained by sowing a suspension of cells on an agar plate. For example, the original D culture was plated on agar and fourteen colonies selected. The yield of each subculture was measured in hundredths of a cubic centimeter per 10 cc. of medium, according to the technique already described (10). All fourteen subcultures yielded more than 6.0.

The original D culture was also placed on gypsum, and two 4-spored and three 1-spored asci were isolated. The two 4-spored asci were dissected and the spores planted separately in culture tubes. Only three of the ascospores from the 4-spored asci produced cultures, while all three 1-spored asci germinated and each produced a viable culture. The yield of the cultures originating from the 1-spored asci approximated that of the single colonies selected by plating vegetative cells. This means that 1-spored asci are genetically similar to the vegetative cells from which they arise. The yield of the single ascospore cultures from 4-spored asci is variable, indicating that the original culture is heterozygous for the factors affecting yield.

The second generation originated from culture Db, which gave a high yield and produced viable 4-spored asci on gypsum. Only one of eight 1-spored asci failed to germinate, and the yield of the 1-spored ascus cultures compared favorably with that of the cultures from which they originated. Fourteen 4-spored asci were dissected and the fifty-six single spores isolated; thirty-three spores produced viable cultures. Both yield and ascospore production were rather variable among these cultures. Only two of the thirty-three cultures produced 4-spored asci; the others generally produced 1- or 2-spored asci.

Sixteen intact 4-spored asci were isolated and planted in hanging drops. All germinated to produce cultures. The resulting colony was suspended in water and plated on agar. Individual colonies were selected and tested for yield. Although only 19% of single ascospore cultures gave a yield of 6.0 or more, 45% of the cultures from self-fertilized asci fell in this category. The following shows the data:

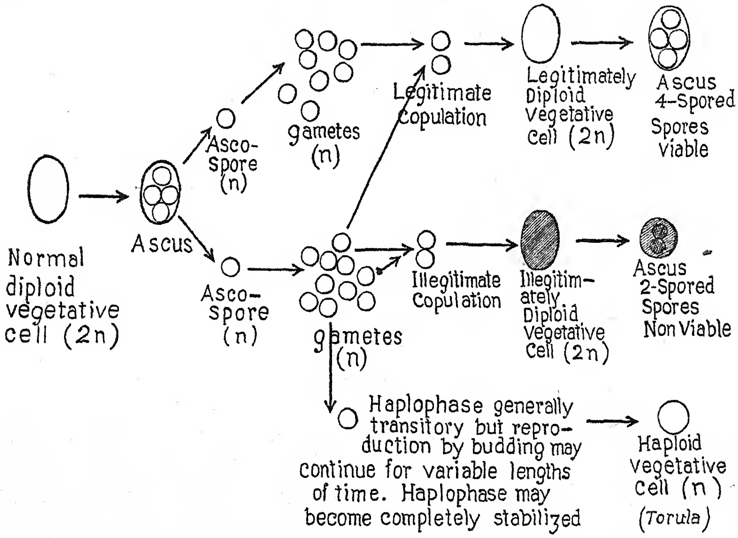

FIG. 1.—Haplophase and diplophase in *Saccharomyces cerevisiae*

Class range	Single ascospore cultures	Colonies from self-fertilized asci
4.0–4.4	6	1
4.5–4.9	10	3
5.0–5.4	23	17
5.5–5.9	42	34
6.0–6.4	16	34
6.5–6.9	3	11

The highest-yielding colonies from the self-fertilized asci were placed on gypsum, and most of them produced an abundance of spores. Three of them (Db5d, Db5h, and Db51) produced viable spores and were used to propagate the third generation.

Only two of the single ascospore cultures in the second generation (Db1A and Db2A) produced 4-spored asci. A total of five 4-spored asci from these cultures were isolated but no cultures were obtained. Sixteen 1-spored, eleven 2-spored, and four 3-spored asci also failed to produce cultures. Some of the asci from single ascospore culture Db25B were viable.

Only twelve cultures were obtained from ninety-four spores originating from single ascospore cultures. In contrast, thirty-five cultures were obtained from seventy-one ascospores isolated from cultures arising from self-fertilized asci. Many unpublished corroborative data have now been accumulated, and the difference between the viabilities of spores arising from single ascospores and from intact asci is much greater than these early data indicate.

One of the self-fertilized asci from culture Db5h (Db5h5b) produced viable 4-spored asci and was carried into the fourth generation. Culture Db512a also produced viable 4-spored asci and was propagated into the fourth generation.

In the pedigree of the D family the only vigorously viable 4-spored asci were obtained from those self-fertilized, and cultures from this source were generally more vigorous as to yield and ascospore viability than cultures from single ascospores. On this basis it seems possible to distinguish two kinds of yeast cultures, those arising from single ascospores and those arising from single asci. This observation has proved useful in the analysis of various commercial baking yeasts.

Sporulation of commercial baking yeasts

Table 1 shows percentages of sporulation and the types of asci found in forty baking yeasts which had been grown on grape-apricot medium and transferred to gypsum slants. The cultures designated

TABLE 1

VARIATION IN ASCOSPORE PRODUCTION OF COMMERCIAL YEASTS

Serial no.	Cells sporulating (%)	Type of ascus	Remarks
B	0.5	1, 2	
LR	5.0	1, 2, 3	
NG4A	0.5	1, 2	
R	0.1	1, 2	
379	2.0	1, 2	
795	0.1	1, 2	
796	5.0	1, 2	
797	2.0	1, 2	
798	1.1	1, 2	
805	Very rare	1, 2	
807	0.5	1, 2	Many 3's
808	5.0	1, 2, 3	
809	0.0	....	
810	0.0	....	
811	5.0	1, 2	
813	1.0	1, 2	
814	10.0	1, 2	
815	5.0	1, 2	
817	15.0	1, 2	
818	0.3	1, 2, 3	Mostly 3's
822	0.0	....	
823	5.0	1, 2, 3	
824	0.01	1	
826	Very rare	1, 2	
BE	5.0	1, 2, 3, 4	Few 4's
D	25.0	1, 2, 3, 4	5% 4's
F	50.0	1, 2, 3, 4	5% 4's
G	30.0	1, 2, 3, 4	20% 4's
HD	10.0	1, 2, 3, 4	10% 4's
LM	40.0	1, 2, 3, 4	5% 4's
M	30.0	1, 2, 3, 4	5% 4's
794	30.0	2, 3, 4	Some 4's
799	20.0	2, 3, 4	
800	30.0	2, 3, 4	Some 4's
801	5.0	1, 2, 3, 4	
806	10.0	2, 3, 4	
812	20.0	2, 3, 4	Some 4's
816	12.0	1, 2, 4	
820	12.0	1, 2, 3, 4	
825	7.0	1, 2, 3, 4	

FIRST GENERATION

Dissected

4-Spored asci

	Yield
D1A	4.0
D1B	1.3
D2B	5.5

1-Spored asci

	Yield
D3A	6.7
D4A	6.0
D5A	6.0

Single colonies

	class range 6.0/6.4	6.5/6.9	7.0/7.4
Da to Dn	3	10	1

Selected colonies

	Yield
Db	6.8
Df	6.5
Dm	6.8

SECOND GENERATION

1-Spored asci

	Yield
Db 33A	5.5
Db 34A	6.2
Db35A	6.9
Db36A	n.g
Db38A	6.7
Db39A	6.8
Db40A	6.3
Db41A	6.2

4-Spored asci dissected

	Yield	Spores
Db1A	6.5	15%
Db1B	6.0	15%
Db1C	4.5	0
Db2A	4.1	5%
Db2B	6.0	5%
Db3A	4.0	
Db17A	5.5	40%
Db17B	5.0	rare
Db17C	5.8	5%
Db17D	5.5	5%
Db18A	5.5	0
Db19A	6.0	50%
Db19B	5.8	50%
Db19C	5.9	30%
Db19D	5.9	rare
Db20A	5.8	70%
Db20B	5.1	2%
Db20C	5.8	10%
Db21A	4.8	
Db21B	4.8	
Db21C	5.8	40%
Db21D	5.1	5%
Db22C	5.2	0
Db23A	5.0	20%
Db23B	5.3	20%
Db23C	5.8	30%
Db23D	5.9	20%
Db25B	6.4	60%
Db25C	5.3	rare
Db25D	6.0	40%
Db26C	6.0	
Db37A	5.4	rare
Db37B	5.6	rare
Db24	No spores germinated	

4-Spored asci Selfed

Ascus	Colonies	class range 4.0/4.4	4.5/4.9	5.0/5.4	5.5/5.9	6.0/6.4	6.5/6.9
Db5	a-i			1	1		3
Db6	a-j			5	5		
Db7	a-i			1	7	1	
Db8	a-h			2	4	2	
Db9	a-h			4	2	2	
Db10	a-g				1	5	
Db11	a-h		1			7	
Db12	a-h		1	2	3	2	
Db13	a-g				4	3	
Db14	a-h			2	1	4	1
Db15	a-h			1	2	5	
Db16	a-h	1			2	3	1
Db28	a-p			1	5	4	6
Db29	a-s			3	6	2	3
Db30	a-n		2	1	7	4	
Db31	a-s		1	2	3	7	3

Selected Colonies

	Yield	Spore	Remarks
Db5d	6.7	80%	viable spores
Db5h	6.8	70%	viable spores
Db5i	6.5	70%	viable spores
Db11d	6.6	50%	small spores
Db14a	6.5	1%	
Db28h	6.5	50%	spores non-viable
Db29k	6.9	60%	spores poorly viable
Db29l	6.5	40%	
Db31b	6.9	50%	
Db31i	6.9	70%	
Db31k	6.7	95%	Spores non-viable

FIG. 2

THIRD GENERATION

Db5d

4-Spored Asci Dissected

Db5d1A	5.5	gray
Db5d1B	5.6	gray
Db5d1C	5.4	gray
Db5d14	no spores germinated	
Db5d15	no spores germinated	
Db5d16A	4.5	gray
Db5d16B	5.4	gray
Db5d17A	4.5	gray
Db5d18A	4.5	gray
Db5d18B	5.8	gray
Db5d18C	4.7	gray
Db5d18D	lost	----
Db5d19A	5.0	gray
Db5d19B	lost	----
Db5d21A	5.5	gray
Db5d21B	5.1	gray
Db5d21C	5.7	gray

12 4-Spored asci selfed and formed colonies
Discarded because of gray color

Db1A

12 1-spored asci n.g
1 4-spored asci n.g

Db2A

4 1-spored asci n.g
11 2-spored asci n.g
4 3-spored asci n.g
4 4-spored asci n.g

Db25B

4 3-spored asci selfed germinated
4 3-spored asci dissected n.g

Db5h

4-Spored Asci Dissected

Db5h1A	5.7	
Db5h1B	5.6	
Db5h6A	5.6	
Db5h6B	6.2	
Db5h6C	3.2	dark
Db5h7A	5.6	
Db5h7B	5.0	
Db5h7C	3.5	
Db5h8A	5.4	
Db5h8B	5.0	gray rough
Db5h9A	5.4	
Db5h9B	5.0	gray rough
Db5h10A	5.6	
Db5h11A	6.3	

4-Spored Asci Selfed

Ascus	Colonies	Class Range 5.5/5.9	6.0/6.4	6.5/7.0	7.0/7.4	7.5/8.0
Db5h2	a-n	1	10	3		
Db5h3	a-n	5	9			
Db5h4	a-p		12	4		
Db5h5	a-o			9	7	1

Colonies Selected	Yield	Spores	Selected Remarks
Db5h3c	6.0	20%	1,2
Db5h3e	6.0	20%	1,2
Db5h3j	6.0	80%	1,2
Db5h4c	6.8	3%	1,2 Small
Db5h4d	6.3	10%	1,2,3 Small
Db5h4j	6.6	30%	1,2,3 Small
Db5h4o	6.5	20%	Small
Db5h5b	6.8	50%	many 4's
Db5h5g	7.0	70%	1,2
Db5h5i	6.8	50%	1,2,3
Db5h5m	6.8	70%	2,3
Db5h5o	7.6	50%	2,3

Db5i

4-Spored Asci Dissected

Db5i1A germinated

Random Ascospores from ruptured asci

	Yield	
Db5i6E	5.5	
Db5i6F	5.5	
Db5i6G	5.0	
Db5i6H	5.0	
Db5i6I	6.2	
Db5i6J	5.6	
Db5i6K	4.0	gray
Db5i6L	n.g	
Db5i6M	n.g	
Db5i6N	n.g	
Db5i6O	n.g	
Db5i6P	n.g	

4-Spored Asci Selfed

Ascus	Colonies	Class Range 5.0/5.4	5.5/5.9	6.0/6.4	6.5/6.9	7.0/7.4
Db5i2	a-l				2	10
Db5i3	a-o	3	1	8	3	
Db5i4	a-h		2	10		
Db5i5	a-m	1	9	2		

Colonies Selected	Yield	Spores
Db5i2a	50%	misshapen
Db5i2i	50%	misshapen
Db5i2j	50%	3's only

FOURTH GENERATION

Db5h5b

4-spored asci dissected

Spore	Yield
Db5h5b1A	5.0
Db5h5b1B	5.0
Db5h5b1A	5.3
Db5h5b2B	5.6
Db5h5b2C	5.9
Db5h5b2A	5.1
Db5h5b3A	5.3
Db5h5b4B	5.5
Db5h5b4C	5.5

4-spored asci selfed

Ascus	Colonies	Class Range 4.0/4.4	4.5/4.9	5.0/5.4	5.5/5.9	6.0/6.4	6.5/6.9	7.0/7.5
Db5h5b5	a-i				3	6	1	
Db5h5b8	a-j	1			4	4		
Db5h5b11					1	3	1	1
Db5h5b12				4	2			

Db5h5b6, Db5h5b7, Db5h5b9, Db5h5b10 } all germinated. No selections made

Db5i2a

4-spored asci dissected

	Yield
Db5i2a1A	5.0
Db5i2a1B	5.5
Db5i2a1C	5.3
Db5i2a2A	5.0
Db5i2a2B	5.0
Db5i2a2C	5.0
Db5i2a2D	5.2

4-spored asci selfed

Db5i2a3, Db5i2a4, Db5i2a5, Db5i2a6 } all germinated. No selections made

FIG. 2.—*Continued*

by letter are commercial bakers' yeasts collected on the market and those designated by number are commercial bakers' yeast cultures obtained from Dr. L. J. WICKERHAM of the Northern Regional Laboratory of the U.S.D.A. at Peoria, Illinois. The percentages of cells which sporulated were usually estimated by inspection. Only sixteen cultures contained 4-spored asci, and only in the most favorable conditions were as many as 5 or 6% of the vegetative cells transformed into 4-spored asci.

Numerous duplicate tests indicate that, in spite of the considerable variation encountered, it is fairly easy to distinguish cultures which sporulate well from those which sporulate poorly. Generally 4-spored asci are found only in those cultures in which almost 20% of the vegetative cells are transformed into asci. In all cultures there were more asci in the 2-spored category than in any other.

It is clear that the cultures fall into two categories, (*a*) those which sporulate poorly and do not produce 4-spored asci and (*b*) those which sporulate relatively well and produce 4-spored asci. On the basis of the data obtained in the pedigree of the D family, the conclusion that the former cultures originated from single ascospores and the latter from self-fertilized asci seems inescapable.

SPORULATION OF R-STRAIN SUBCULTURES

Thirty-one subcultures were obtained by plating out the R-strain culture, and each was tested for ascospore production (table 2). They all fall into the poorly sporulating category, with only a few rare 4-spored asci. Subcultures of a poorly sporulating culture rarely show improvement over the parent strain, but it is clear from the pedigree of the D family that subcultures of a vigorously sporulating culture are often extremely inferior to the parent in spore-producing ability.

TABLE 2

SPORULATION IN CULTURES ISOLATED FROM R STRAIN

Serial no.	Cells sporulating (%)	Type of ascus	Remarks
Ra	2.0	1, 2	
Rb	1.0	1, 2	
Rc	0.5	1	
Rd	0.5	2, 3	Small
Re	3.0	1, 2, 3	
Rf	5.0	1, 2	
Rg	0.5	1, 2	
Rh	0.5	1, 2	Small
Ri	1.0	1, 2, 3	Small
Rj	0.5	1, 2	
Rk	0.5	1, 2	
Rl	0.5	1, 2	
Rm	5.0	1, 2, 3	Large
Rn	0.5	1, 2	
Ro	1.0	1, 2	
Rp	1.0	1, 2	
Rq	2.0	1, 2, 3	
Rr	5.0	1, 2, 3, 4	Large
Rs	5.0	1, 2	
Rt	1.0	1, 2	
Ru	Rare	1, 2	
Rv	2.0	1, 2	
Rw	3.0	1, 2	
Rx	3.0	1, 2	Small, irregular
Ry	0.5	1, 2	Small, irregular
Rz	7.0	2, 3	Large spores
Raa	1.0	2, 3, 4	
Rba	5.0	1, 2, 3	
Rca	2.0	1, 2	
Rda	2.0	1, 2, 3, 4	
Rea	2.0	1, 2, 3, 4	

Individual 2-, 3-, or 4-spored asci from various R subcultures were selected and planted on hanging drops of agar in a moist chamber with a micromanipulator. It was necessary to search diligently to obtain any 3- or 4-spored asci, although in some cultures 1- and 2-spored ones were rather easy to find. Most of the spores failed to germinate. Some of them germinated but produced only a small

cluster of the round haploid cells, and these all died. Transfer to a fresh culture tube failed to produce a colony. Only one viable colony was obtained from forty-seven 2-spored asci.

TABLE 3

NUMBER OF VIABLE COLONIES OBTAINED FROM 1-, 2-, 3-, AND 4-SPORED ASCI OF CULTURES ORIGINATING FROM R STRAIN

Subculture	Type of ascus	No. of asci	Viable colonies
Rda	1	4	3
Rda	2	4	0
Rda	4	1	0
Raa	1	8	4
Rz	2	4	0
Rz	3	4	1
Rea	1	4	3
Rea	2	33	1
Rea	4	1	0
Rt	2	6	0
Rt	4	1	0

In contrast to the 2-, 3-, and 4-spored asci, the 1-spored were much more viable and germinated differently. The viable spores from 1-spored asci germinated directly to produce large ellipsoidal diploid cells, instead of the small round haploid cells characteristic of the ascospores from the 2-, 3-, and 4-spored asci. Ten of sixteen spores from 1-spored asci produced living colonies (table 3).

The R-strain culture obviously has all the characteristics of a single ascospore culture—poor sporulation, low viability, and predominantly 2-spored asci. Diligent efforts to select a subculture with a superior ability to produce spores yielded only negative results.

Summary

1. Sporulation in *Saccharomyces cerevisiae* is influenced by the genetic constitution of the culture as well as by the composition of the nutrient medium. A specific nutrient medium which gives maximum sporulation has been developed and described.

2. On this medium, heterozygous, legitimately diploid cultures produce an abundance of viable 4-spored asci. Single ascospore cultures (which may be either illegitimately diploid or haploid) sporulate much more irregularly; some do not sporulate at all. The asci from single ascospore cultures generally contain only one or two spores, and their viability is much diminished.

3. A survey of forty commercial baking yeasts was made. Some were legitimately diploid, while others were of single ascospore origin.

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LITERATURE CITED

1. BALTATU, E., Mycoderma als echte *Saccharomycetes*. Cent. Bakt. II. 101:196–225. 1939.
2. BEYERINCK, M. W., Schizosaccharomycetes ochtosporous, eine achtsporige Alkohol hefe. Cent. Bakt. I. 16:49–58. 1892.
3. ———, Ueber Regeneration der Sporenbildung wo diese Funktion im Verschwinden begriffen ist. Cent. Bakt. II. 4:657–663; 721–730. 1898.
4. ENGEL, L., Les Ferments alcooliques. University of Paris. 1–59. 1872.
5. GORODKOWA, A. A., Ueber das Verfahren rasch die Sporen von Hefepilzen zu gewinnen. Bull. Jard. Imp. Bot. St. Peters. 8:163–170. 1908.
6. GRAHAM, V. R., and HASTINGS, E. G., Studies on film-forming yeasts. Canad. Jour. Res. 19:251–256. 1941.
7. HANSEN, E., Recherches sur la physiologie et la morphologie des ferments alcooliques. II. Les ascospores chez le genre *Saccharomyces*. Compt. Rend Lab. Carls. 2:29–85. 1883.
8. KRUIS, K., and ŠATAVA, J. O. vývoji a klíčení spór jakož i sexualitě kvasinek. Nakl. Č., Akad. Praha. 1918.
9. LINDEGREN, C. C., and HAMILTON, ELIZABETH, Autolysis and sporulation in the yeast colony. BOT. GAZ. 105:316–321, 1944.
10. LINDEGREN, C. C., and LINDEGREN, GERTRUDE,

Environmental and genetical variations in yield and colony size of commercial yeasts. Ann. Mo. Bot. Gard. 30:71–82. 1943.
11. ———, and ———, Selecting, inbreeding, recombining and hybridizing commercial yeasts. Jour. Bact. 46:405–419. 1943.
12. MANEVAL, W. E., A method of securing spores of yeasts. BOT. GAZ. 78:122–125. 1924.
13. MRAK, E. M., PHAFF, H. J., and DOUGLAS, H. C., A sporulation stock medium for yeasts and other fungi. Science 96:432. 1942.
14. NICKERSON, W. J., and THIMANN, K. V., The chemical control of conjugation in *Zygosaccharomyces*. Amer. Jour. Bot. 28:617–621. 1941.
15. ———, and ———, The chemical control of conjugation in *Zygosaccharomyces*. Amer. Jour. Bot. 30:94–101. 1943.
16. REES, M., Zur Naturgeschichte der Bierhefe, *Saccharomyces cerevisiae* Meyen. Bot. Zeit. 27: 106–118. 1869.
17. ŠATAVA, J., O Redukovaných Formách Kvasinek. V. Praze Nakladem Vlastnim. 1918.
18. ———, Les formes sexuelles et asexuelles des levures et leur pouroir fermentatif. III. Cong. Internat. Tech. Chim. Ind. Agr. Paris. 1934.
19. SEYNES, M. J. DE, Sur le mycoderma vini. Compt. Rend. Acad. Sci. 67:105–109. 1868.
20. WELTEN, H., Wan bildet die Hefe Sporen? Mikrokosmos 8:3–5; 41–43. 1914.
21. WINGE, O., On haplophase and diplophase in some *Saccharomyces*. Compt. Rend. Lab. Carls. Serie Physiol. 21:77–112. 1935.

AUTOLYSIS AND SPORULATION IN THE YEAST COLONY[1]

CARL C. LINDEGREN AND ELIZABETH HAMILTON

Knowledge of life-cycles and variations in bacteria has developed almost exclusively from the study of isolated individual cells, without consideration of the structural organization of the colony. LEGROUX and MAGROU (8) studied the structure of colonies of *Vibrio cholerae* and of a number of other bacteria. They discovered that rod-shaped variants of the *Vibrio* appear in perpendicularly arranged packets on the exterior of the colonies, held together by a transparent substance apparently exuded from the cells. They assumed that this substance gave the rough colonies their rigidity. A special stain was used:

Eosinate of methylene blue	7.0 gm.
Eosinate of toluidin blue	1.5
Toluidin blue	5.0
Methyl alcohol (99.5%)	490 cc.

The rod-shaped organisms in the outer layer of the colony were made up of a violet central region and an azure exterior. The central region underwent division into two or four rounded violet particles, often of unequal size. This phenomenon was found in *Vibrio*, in the typhoid bacillus, in the diphtheria bacillus, and in the tubercle bacillus. KAHN (7), apparently unacquainted with the work of LEGROUX and MAGROU, confirmed their findings in a study of the tubercle bacillus but concluded that the outer layer was the younger part of the colony. The work of LEGROUX and MAGROU proves this view untenable.

PISOVA (18) found that, when a yeast colony grows on agar, a pseudo-mycelial growth of long fibrous cells penetrates the agar, especially at a high sugar concentration. After a few days or weeks, the surface cells begin to autolyze, continuing until an outer layer of autolyzed cells is formed.

Experimentation

After yeast colonies had been grown on malt-yeast agar,[2] portions of the agar containing colonies were cut out and dropped into Flemming's solution. After

[1] This work was supported by a grant from Anheuser-Busch, Inc., St. Louis.

[2] Malt extract 10.0%, dextrose 0.5%, dried brewers' yeast 0.5%, agar 3.0%, $CaCO_3$ 1.0%.

24 hours the killing fluid was washed out in tap water, and the material was imbedded in paraffin. Sections were made and stained with a variety of dyes. Direct smears were also made by cutting the fresh colony in half vertically with a razor and pressing the exposed section gently against the slide. The cells were fixed on the slide by heat and stained.

Figure 1*A* shows a section of the yeast colony. The outer layer of autolyzed cells stains very lightly, and the inner central mass of vegetative cells with their dense protoplasts is much darker. A pseudo-mycelium of yeast cells penetrates the agar and is thickest and deepest at the edges of the colony, apparently where oxygen is most abundant. The thin peripheral film of cells at the edge of the colony spreads over the surface of the agar. Figure 1*B* shows details at the ends of the section, with cells penetrating the agar. At the points of origin of penetration the cells are very abundant. This may be due to the channeling of the substrate nutrient into these regions along the cracks made by the pseudo-mycelium.

Figure 2 shows the central vegetative cells, many of which are extremely small, indicating that cell division continued after the nutrients became less readily available and competition resulted in a decrease in cell size. Figure 3 shows the pseudo-mycelium produced in the agar in higher magnification.

Figures 4 and 5 illustrate the autolyzed layer containing the asci. Only a few cells produce asci. Autolysis apparently occurs early in the history of the colony, at least before competition reduces cell size. The autolyzed layer contains the only asci found, suggesting that autolysis supplies essential nutrients on which sporulation depends. NICKERSON and THIMANN (16) reported a correlation in *Zygosaccharomyces* between the number of dead cells and the number of cells copulating and proposed that "stimulating" substances were released by the dead cells which cause those still living to conjugate and to sporulate. Later (17) they showed that riboflavin and sodium glutarate were specific substances which promoted copulation and sporulation. MANEVAL (15) pointed out that spores appear on the outside of a stored yeast cake.

In some of the contact smears the autolyzed cells were not so shrunken as those obtained by the paraffin method. The walls seemed relatively intact, but there were no stainable cell contents. The "ghost" cells were larger than the densely stained cells in the vegetative section of the colony. These autolyzed cells, which apparently serve as sources of nutrients for the sporogenous cells, have a parallel in the paraphyses found in Pyrenomycetes and Discomycetes which also act as nurse cells. In some regions of the autolyzed layer, small clusters of round, apparently haploid, cells were found, suggesting that some spores may germinate in the layer. New genetic rearrangements could result from such spore germination and subsequent copulation. These experiments suggested that yeast autolysate is an essential nutrient for sporulation and led to the addition of 2% dried brewers' yeast to a pre-sporulation medium, with excellent results (14).

Discussion

The striking parallelism between the structure of yeast colonies and those of bacteria, as shown by LEGROUX and MAGROU, suggests that the life-cycles may be similarly parallel. Yeasts possess a proved nuclear mechanism, and the vegetative cells undergo meiosis and

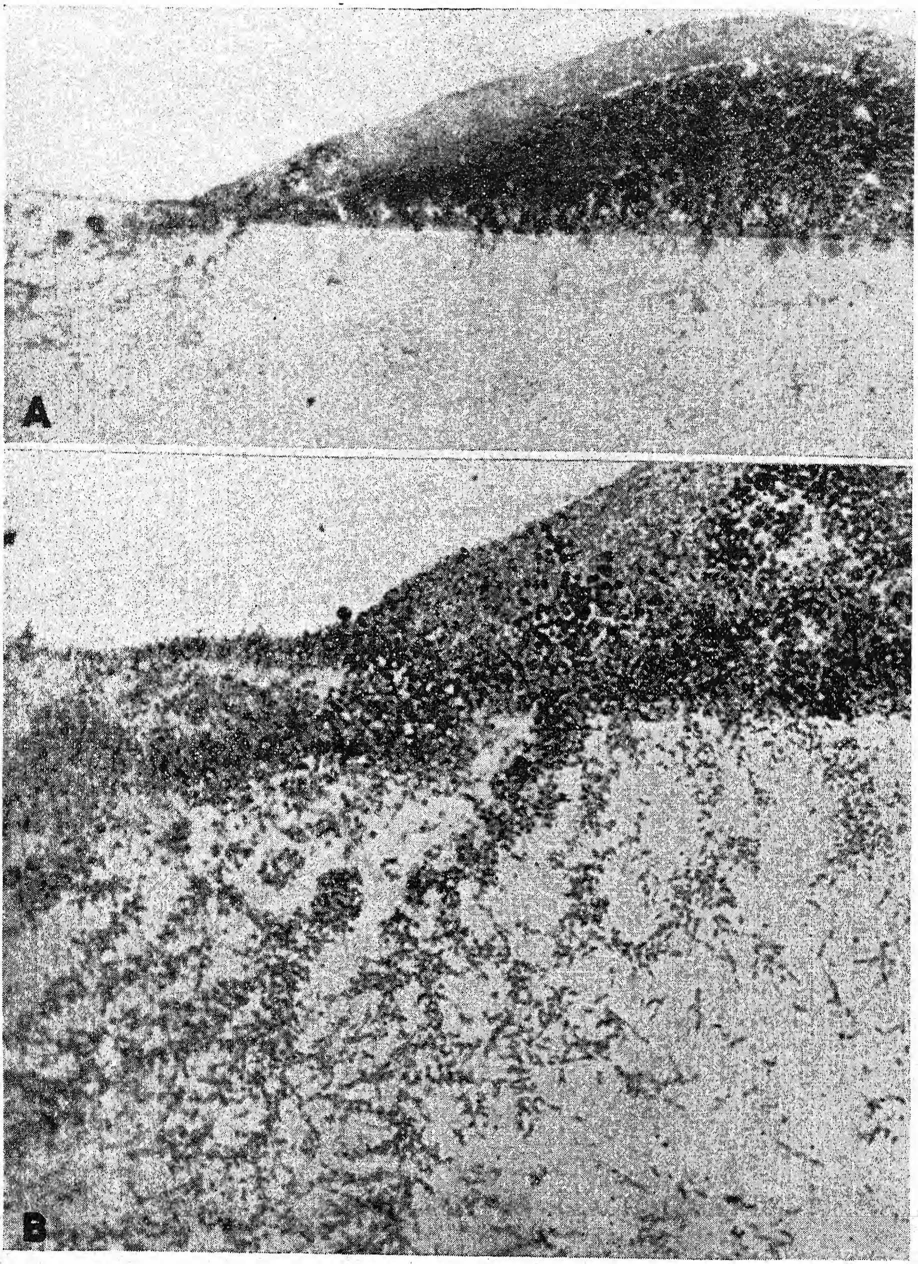
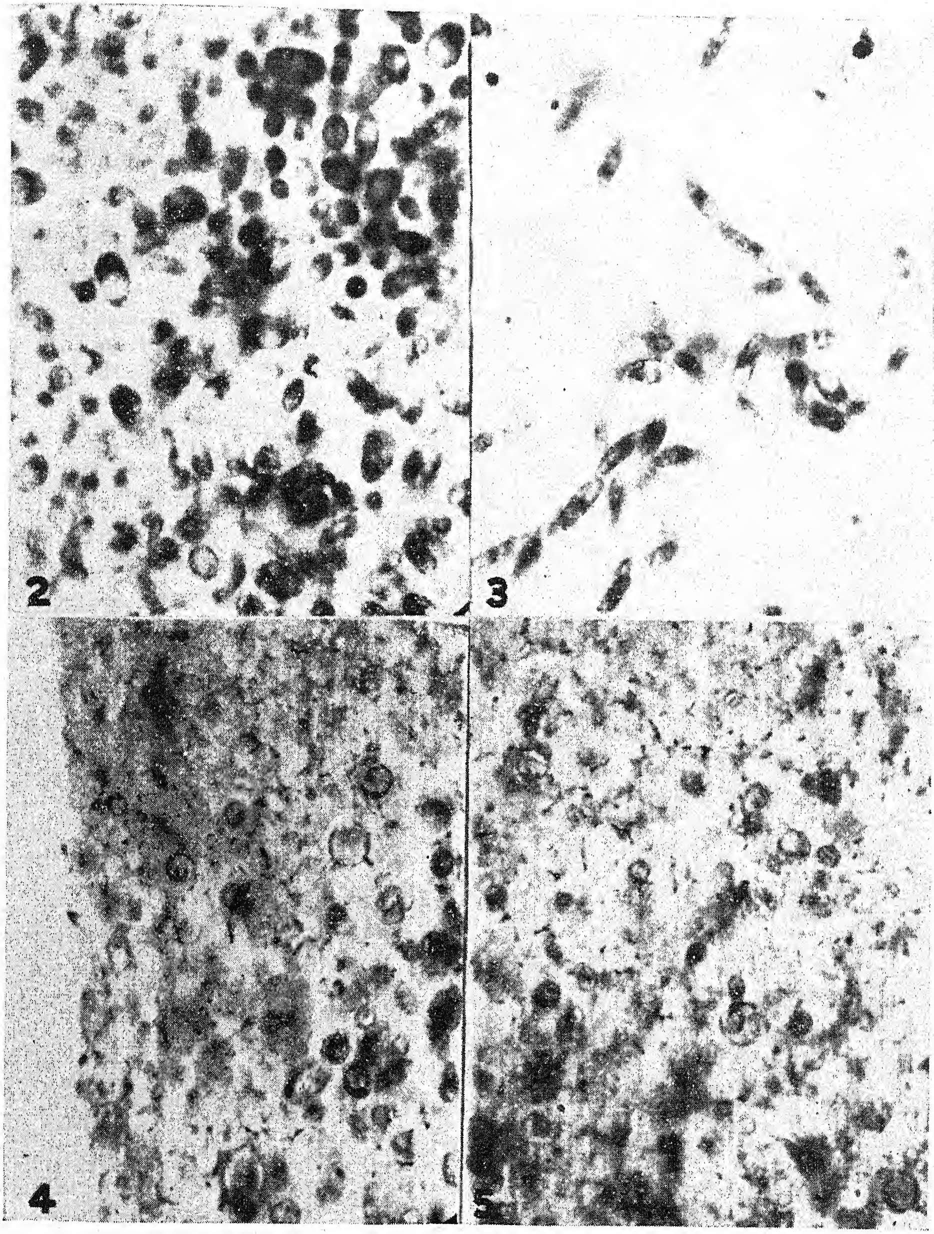


FIG. 1.—*A*, cross-section of yeast colony showing outer autolyzed layer, central region containing vegetative cells, and elongate pseudo-mycelial cells penetrating agar. *B*, left end of *A*, under higher magnification, showing penetration of agar by the pseudo-mycelium.



FIGS. 2–5.—Fig. 2, central region of vegetative cells in yeast colony. Neither spores nor autolyzed cells present but many small cells apparently produced by continued division in region of diminished nutrient. Fig. 3, higher magnification of pseudo-mycelium in agar. Figs. 4, 5, highly magnified outer layer of colony. Most cells autolyzed and walls disintegrated; those not autolyzed have sporulated and become asci. Many contain one and two spores; one 4-spored ascus visible.

sporulation in the outer layer of the colony. In bacterial colonies a similar division of the cell contents into two or four bodies occurs in the corresponding layer. The analogy suggests strongly that the granulation of the bacterial cell is the result of a reduction division similar to that known to occur in yeasts. The cytological analyses of BADIAN (1–5) and LINDEGREN (9–13) have shown that chromosomes exist in bacteria and that they undergo a series of changes comparable with those observed in the reduction divisions of higher plants. Using the same technique as developed in his study of bacteria, BADIAN (6) made a complete study of the chromosomal mechanism of several species of yeast. He found that all contained two chromosomes in the haplophase which fused end to end (presumably at the chromomeres) to produce two chromosomes in the diplophase. Reduction occurred at spore formation. Dr. HAMPTON CARSON of Washington University has confirmed the fact that yeasts contain two chromosomes in the diplophase, by using the Feulgen stain. It would seem that the work of BADIAN should supersede all earlier descriptions of chromosomes in yeast. The accuracy of his cytological findings in yeasts tends to confirm his descriptions of reduction divisions in bacteria, which suggest that spore and granule formation in the bacteria may be homologous to ascospore formation in yeasts.

Summary

Yeast colonies contain an outer layer of autolyzed cells, and this layer is the region in which sporulation occurs. Autolysis of yeast cells probably provides a substrate favorable for sporulation. LEGROUX and MAGROU showed that in bacterial colonies a similar structural (one might almost say histological) differentiation exists. The bacterial cells in the outer layer of the colonies produced tetrads, which may possibly be homologous to the ascospores of yeasts.

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LITERATURE CITED

1. BADIAN, J., Z Cytologji Miksobakteryj. Acta Soc. Bot. Pol. 7:55–71. 1930.
2. ———, Eine cytologische Untersuchung ueber das Chromatin und den Entwicklungszyklus der Bacterien. Ark. Mikro. 4:409–418. 1933.
3. ———, Eine cytologische Untersuchung ueber die *Mixobacterie polyangium fuscum*. Acta Soc. Bot. Pol. 10:361–378. 1933.
4. ———, Sur la Cytologie du *Bacillus megatherium*. *Ibid.* 13:69–74. 1935.
5. ———, Ueber die cytologische Structur und den Entwicklungszyklus der Actinomyceten. *Ibid.* 13:105–126. 1936.
6. ———, Sur la cytologie des levures. Bull. Acad. Pol .Sci. Lett. Ser. B. Science Nat. 50:61–87. 1937.
7. KAHN, M. C., and NONIDEZ, JOSÉ, Non-acidfast rods and granules in vertical sections of *Mycobacterium tuberculosis* colonies. Proc. Soc. Exp. Biol. Med. 30:577–582. 1933.
8. LEGROUX, RENÉ, and MAGROU, J., État organisé des colonies bacteriennes. Ann. Inst. Past. 34: 417–431. 1920.
9. LINDEGREN, C. C., and MELLON, R. R., Nuclear phenomena suggesting a sexual mechanism for the tubercle bacillus. Proc. Soc. Exp. Biol. Med. 30:110–112. 1932.
10. LINDGREN, C. C., Genetical studies of bacteria. I. The problem of the bacterial nucleus. Cent. Bakt. II. 92:41–48. 1935.
11. ———, Genetical studies of bacteria. II. The problem of bacterial variation. *Ibid.* II. 92: 113–122. 1935.
12. ———, Genetical studies of bacteria. III. Origin of G-type colonies by transgenation. *Ibid.* II. 92:389–400. 1935.

13. ———, Nuclear apparatus and sexual mechanism in a micrococcus. Iowa State Coll. Jour. Sci. 16:307–318. 1942.

14. Lindegren, C. C., and Lindegren, Gertrude, Sporulation in *Saccharomyces cerevisiae*. Bot. Gaz. 105:304–316. 1944.

15. Maneval, W. E., A method of securing spores of yeast. Bot. Gaz. 78:122–125. 1924.

16. Nickerson, W. J., and Thimann, K. V., The chemical control of conjugation in *Zygosaccharomyces*. Amer. Jour. Bot. 28:617–621. 1941.

17. ———, The chemical control of conjugation in *Zygosaccharomyces*. Amer. Jour. Bot. 30:94–101. 1943.

18. Pisova, Mildred, Anatomy of yeast colonies. Unpublished thesis. Karl University, Prague. (Additions by Dr. Němeč. 1930.)

PHOTOPERIODICITY OF BAERIA CHRYSOSTOMA

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Introduction

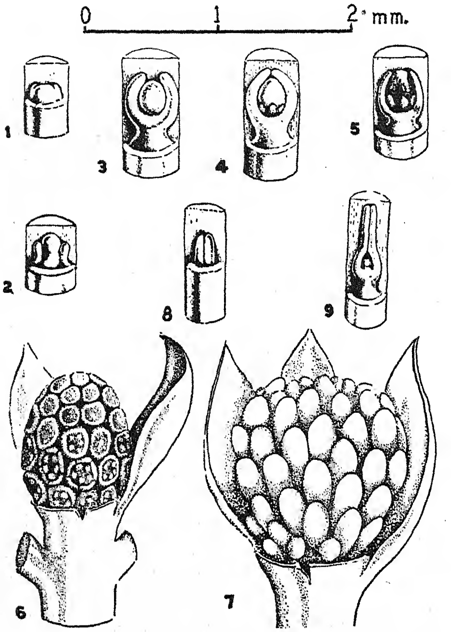
Most desert annuals in California flower in the same month each year (in early spring), irrespective of when germination took place. In years with early winter rains, germination occurs early and the annuals reach considerable size before they start to flower. When the rains come late in winter, the annuals have only a short period of vegetative development and flower while still small. This indicates that they may be long-day plants. Preliminary experiments with about eighty of these annuals showed that many of them actually are long-day plants and do not flower in a daily 8-hour photoperiod but do flower when the 8-hour photoperiod is supplemented with 8 or 16 hours of artificial light. The plants which grew best and flowered first under controlled conditions and artificial light were *Baeria chrysostoma* (minimum of 19 days between germination and anthesis) and *Phacelia parryi* (minimum of 23 days). Both plants are small and therefore can be grown in great numbers in a limited space (one plant per 2 cm.^{2}), making them excellent experimental material.

Methods.—Seeds of *Baeria chrysostoma* and *Phacelia parryi* were obtained from the Rancho Santa Ana Botanic Garden, through the courtesy of its Director, Mrs. S. Bixby Bryant. The seeds were sown in washed and sterilized silica sand in small glass cylinders, 4 × 8 cm., with open bottoms for drainage. They were covered with sand and after germination were thinned to about five plants per cylinder. The seeds were watered with Hoagland's nutrient solution once every 2 days. By not watering too often and by sterilizing the cylinders and sand together (20 minutes at 2 atmospheres), damping-off of the seedlings could be controlled. Whenever necessary, the plants were sprayed against aphids with an oil emulsion (Extrax). The plants were all grown in artificial light of about 250 f.c., from fluorescent bulbs in rooms with controlled temperature and humidity (10). As a short photoperiod, exposure of 8 hours daily, usually from 8 A.M. to 4 P.M., was employed throughout the investigation. Except where noted, long photoperiod meant continuous exposure to the artificial light; one long photoperiod was exposure from 8 A.M. one day until 4 P.M. the next day.

Under the conditions of these experiments, the development of *Baeria* is as follows:

The seeds germinate within 24 hours after sowing. In another 24 hours the two cotyledons have appeared. These

are linear and almost cylindrical and in the first few days reach a length of 10–14 mm. The growing point can be seen only by pulling the cotyledons apart. All further observations are best carried out



FIGS. 1–9.—Apical growing points of *Baeria chrysostoma* (front leaf pulled off, leaving semicircular scar): Figs. 1–5, successive stages of flower primordia; in all cases, two youngest opposite leaf primordia are separated by flower primordium, which has just initiated involucral bracts in fig. 4 and florets in fig. 5. Figs. 6, 7, later stages; two highest foliage leaves and 2–3 involucral bracts removed to show flower head. Fig. 8, growing point of purely vegetative plant; only two leaf primordia visible; small apical meristem hidden between them. Fig. 9, growing point kept at 28° C. in continuous light; flower primordium initiated but not developing further and overgrown by leaf primordia.

under a preparation microscope with a 20–40× magnification. Practically the whole growing point differentiates into two opposite leaves, with almost no meristematic tissue between. Soon the first pair of foliage leaves starts to elongate (fig. 8), about 4 days after germination. Almost as soon as the growing point between these leaves enlarges, it divides again into two leaf primordia, with very little meristem between. Simultaneously with the leaf growth, the first internode elongates a few millimeters. This process is repeated as long as the plant remains vegetative, and a succession of opposite linear leaves develops. Only on long photoperiod and high intensities is a narrow leaf lamina formed.

As soon as the plant changes to the flowering condition, the originally hardly visible growing point rapidly widens, resulting in a third cellular dome between the two leaf primordia (fig. 1). At this very early stage of flower differentiation, no mistake can be made between this and the vegetative growing point. Further development of the flower head is shown in figures 2–7. There was a 12-day time difference between figure 1 and figure 5 (plants grown in continuous light). The growing point enlarges to a ball-shape, and then first the involucral bracts and later the individual flowers are differentiated in acropetal succession. The number of flowers per head is variable; any number between seven and fifty may be encountered. Differentiation of the individual flower proceeds as follows: first a ring appears, later giving rise to the corolla (figs. 5, 6, upper flower primordia); then the five stamens are differentiated, and simultaneously the meristematic ring splits into five corolla lobes (fig. 6, lower flowers); further differentiation can be observed only by cutting away the rapidly growing corolla (fig. 7).

After flower initiation, but while the plants are still kept under poor growing conditions (high temperature), the flower primordium does not enlarge further and is being overgrown by the youngest leaf primordia (fig. 9).

In the following descriptions the various stages of flowering are referred to by number, for simple calculation of averages for flower formation. The vegetative stage (fig. 8) is designated as 0. The various stages of flower formation have the same number as that of the figure: the growing point of figure 1 is in stage 1, that of figure 2 is in stage 2, etc. Figure 9 is referred to as stage $\frac{1}{2}$. It is possible to judge miscroscopically a few days after long photoperiodic treatment whether initiation has occurred or not; macroscopically it takes 1–2 weeks longer. In the present experiments floral initiation occurred generally after three pairs of leaves were formed, only rarely after the second pair.

All plants grown under the laboratory conditions of low light intensity remain small and depauperate, when compared with plants growing in nature. Whereas in nature the plants generally branch more or less freely (4, fig. 10*a*), in these experiments branching was not so common. Figure 10*b* shows the general appearance of a *Baeria* plant 25 days old grown in continuous light of 300 f.c.

Results

In preliminary experiments it was found that only when *Baeria* was kept under long photoperiods did flowering occur, irrespective of light intensity. No plants kept daily for 8 hours in normal daylight and 16 hours in darkness showed signs of flowering, either in summer or in winter.

The first experiments were conducted in a room kept continuously at 28° C., and artificial light of about 250 f.c. was used. Both germination and growth were poor under these conditions, and within 40 days all plants died without the formation of flowers, even when kept in continuous light. Microscopic examination showed no floral initiation after 4, 5, 6, or 7 days of continuous light, starting 3 days after germination. The observations were made at intervals of 2 days, starting at 11 days and ending 20 days after the beginning of the treatment. Only occasionally (about 20%) was stage $\frac{1}{2}$ (fig. 9) observed but never any of the stages of flowering of figures 2–7. During this period there was considerable growth of the second pair of leaves (from 2 mm. to 8 mm.).

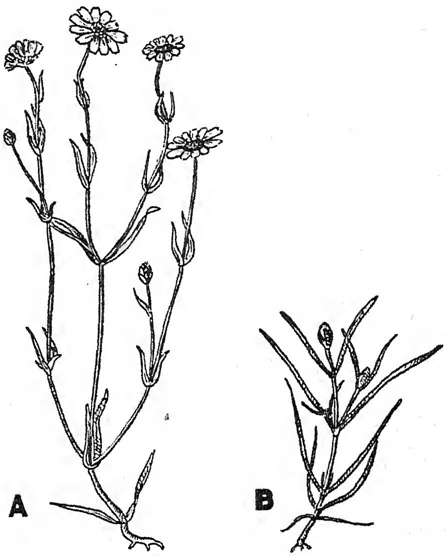


FIG. 10.—*A*, *Baeria chrysostoma* var. *gracilis* after MUNZ (4); *B*, as grown at 22° C. in continuous light, 25 days after germination.

Later it was attempted to give the plants a cold treatment, by leaving them for 1–7 days in a cool room kept at 22° C. day and night, afterward returning them to 28° C. Stage $\frac{1}{2}$ was reached in only 10% of the plants; all others were purely vegetative. In another experiment the plants were kept only during the 16 dark hours at 10° C. and for 8 hours each day at 28° in artificial light. Subsequent long photoperiodic treatment at 28° with 5, 7, and 9 days of continuous light did not result in flowering.

Table 1 gives the data for an experiment in which the temperature was kept at either 28° or 22° C. during the photoperiod. Only those plants which received continuous photoperiod at 22° flowered irrespective of their pre-treatment. Neither short photoperiods at 22° nor long ones at 28° resulted in flowering. All subsequent experiments were therefore carried out at a constant temperature of 22°, with no further work at the higher temperature.

TABLE 1

RELATION BETWEEN TEMPERATURE AND PHOTOPERIOD AS AFFECTING FLOWERING IN BAERIA. ALL PLANTS (7–8 PER TREATMENT) OBSERVED 23 DAYS AFTER GERMINATION. PLANTS SUBJECTED AS INDICATED TO PRELIMINARY TREATMENT FOR 8 DAYS; SUBSEQUENT TREATMENT FOR ANOTHER 8 DAYS; FINAL UNIFORM TREATMENT FOR LAST 7 DAYS AT 22° C. ON SHORT (8-HOUR) PHOTOPERIOD

SUBSEQUENT TREATMENT 8 DAYS \ PRELIMINARY TREATMENT 8 DAYS		8-HOUR PHOTOPERIOD 28° C.	8-HOUR PHOTOPERIOD 22° C.	24-HOUR PHOTOPERIOD 28° C.
8-HOUR PHOTOPERIOD	22° C.	....	....	Vegetative
24-HOUR PHOTOPERIOD	28° C.	....	Vegetative	Vegetative
	22° C.	Flowering	Flowering	Flowering

The number of long photoperiods required to induce flowering was investigated. The plants were germinated and after 5 days were exposed to long photoperiods for 5, 6, 7, 8, 9, or 10 consecutive days. They were then returned to an 8-hour photoperiod. They were observed microscopically either 6 or 13 days after termination of the treatment. After 6 days, the 5, 6, and 7 long photoperiods had not resulted in visible flower differentiation; but in the 8, 9, and 10 long photoperiods, only two out of thirteen plants remained vegetative, six were in stage 1 and five in stage 2 or 3. After 13 days only one plant had remained vegetative, and all twenty-seven others which received 5–10 long photoperiods were in some stage of flower differentiation; the plants kept on 5 and 6 long photoperiods had not progressed as far as the others.

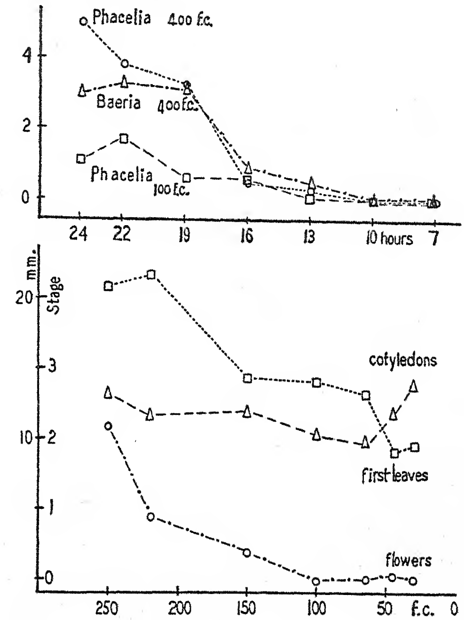
When plants of *Baeria* were exposed to 8 long photoperiods immediately after germination, or 1, 2, 3, or 5 days later, observation 7 days after conclusion of the treatment showed that all plants exposed (starting 5 days after germination) were in an advanced stage of flower differentiation, but in those exposed 0, 1, 2, or 3 days after germination the results were variable: nine plants remained vegetative, nine were in stage 1, and four had progressed to stages 2–3. Apparently the plants become responsive to the long photoperiods very soon after germination. In another experiment, the long photoperiodic treatments were started 4, 7, 10, 13, or 16 days after germination. Whereas after 4 days, 11% of the plants remained vegetative, 9% did not flower when 7–16 days elapsed between germination and exposure to long photoperiods. Therefore the plants have reached full sensitivity to long photoperiods 4 days after germination. In this same experiment it was found that 0, 1, and 2 long photoperiods were completely ineffective in inducing flowering; 8 and 10 long photoperiods gave 100% flowering, 4 long ones gave 38% flowering (stage 1), and 6 long photoperiods gave 79% flowering (stages 1–3). These experiments show that 4 long photoperiods are not quite enough to initiate flowers in 50% of all plants, but 8 such periods are fully effective, whereas further growth of the flower primordia is facilitated by extended exposures to long photoperiods.

To determine the daily photoperiod re-

quired for flower initiation, a long cabinet was constructed which had a sliding cover, by which the plants growing inside the seven compartments of the cabinet could be shut off in succession from the light of fluorescent tubes mounted above the cover. A motor activated by a timeclock pulled this cover back, one compartment at a time. By regulating the timeclock, successive compartments could be exposed to light for successively shorter periods. At a set time the sliding cover was pulled back, so that the plants in any one compartment were exposed to the same photoperiod each day.

In figure 11 the results of one such experiment are shown. Plants of *Baeria* and *Phacelia* were germinated in daylight in a greenhouse. Thirteen days later the plants of *Baeria* had two fully developed first leaves. These plants were then placed in the cabinet at 20° C. constant temperature. Ten days later the plants were observed microscopically and their average flower development expressed in a number corresponding to their stage (ordinate in fig. 11). Whereas there was no trace of flower initiation on the 7- and 10-hour photoperiods, on a 13-hour photoperiod three out of ten plants had reached stage 1, the rest remaining vegetative; on a 16-hour photoperiod only three out of ten plants were still vegetative, whereas on the 19-, 22-, and 24-hour photoperiods flower formation had progressed to stages 2–4 and no vegetative plants were found. Comparable plants, exposed in the greenhouse to the natural 10-hour photoperiod of middle December, were all vegetative, except four out of twenty, which were in stage 1–2. Apparently 15 hours of daylight is the critical photoperiod for *Baeria* at the low light intensities employed. The critical photoperiod for *Phacelia* also is 15 hours (fig. 11), and at lower intensity there is little further development of the flower primordia.

In other experiments, the same 15-hour critical photoperiod was found; it is clear, however, that this critical photo-



FIGS. 11, 12.—Fig. 11 (above), relation between length of photoperiod (abscissa, in hours) and flowering of *Baeria* (triangles) and *Phacelia* (circles, 400 f.c.,; squares, 100 f.c.). Flowering stages plotted on ordinate, o being vegetative. Fig. 12 (below), length of cotyledons (triangles) and first leaves (squares) of *Baeria* plants grown at 22° C. and continuous light of different intensity (abscissa, in foot candles). Also flowering plotted (circles) in terms of stage reached (ordinate, o = vegetative).

period is not as sharply defined as in *Xanthium* (3), but more like that in soybeans (1).

The lowest light intensity which could still induce flower formation was determined. The plants were either grown continuously in different light intensities or kept for 8 hours each day in 250 f.c., being subjected to different intensities for the other 16 hours (table 2). In continuous light an intensity of more than 200

f.c. is required for formation of fully developed flower buds. At lower intensities only a few flowers develop, or none are initiated. At intensities below 150 f.c. vegetative development was very meager.

Figure 12 shows the results of an experiment in which plants of *Baeria* were kept 5–13 days after germination at 300 f.c. for 8 hours each day, and for the remaining 16 hours at different intensities. Then they were returned to an 8-hour photoperiod of 300 f.c. only. Measurements were made 19 and 25 days after germination (6 and 12 days after the end of treatment). Length of the cotyledon was not materially affected by the light. On the other hand, length of the first pair of leaves was much greater at the two highest intensities. Flowering was induced only by exposure to light of more than 100 f.c.; beyond that there was a marked effect of intensity on the further development of the flower primordium.

The effect of different colors of light (all of 250 f.c. intensity, obtained by passing light of a 1000-watt incandescent light through Corning glass filters) when used in addition to a daily 8-hour exposure to 1500 f.c. is shown in table 3. As expected, the controls were completely vegetative but had fairly good leaf development. In green light the plants were also vegetative, and their leaf growth was about the same as the controls kept in darkness. Red was most effective for flowering, then yellow, and blue was least effective. In another experiment the effects of blue, yellow, and red were approximately the same for flowering, but the controls and plants in green light were vegetative.

TABLE 2

FIVE DAYS AFTER GERMINATION, BAERIA PLANTS WERE PLACED CONTINUOUSLY IN DIFFERENT LIGHT INTENSITIES. OBSERVATIONS ON DEVELOPMENT AND FLOWERING WERE MADE AFTER TWO TIME INTERVALS

LIGHT INTENSITY (F.C.)	17 DAYS AFTER GERMINATION					STAGE OF FLOWERS 29 DAYS AFTER GERMINATION
	No. of leaves	Length (mm.)			Stage of flowers	
		Cotyledons	First two leaves	Second two leaves		
250	4	8	13	11	5, 5, 5, 5, 4, 2, 1	7, 7, 7
206	6	12	19	12	6, 5, 4, 3, 3	7, 7, 7
162	4	12	20	5	5, 5, 1, 1	1, 1, 1, 0
118	0	16	< 1	< 1	All 0	5, 1, 1, 1, 0
74	0	15	< 1	....	All 0	1, 1, 0, 0
30	0	11	< 1	....	All 0	0, 0, 0

In an earlier experiment lower intensities (below 100 f.c.) of colored light were used as additional light to an 8-hour photoperiod in white light. As was to be expected, none of the colors induced flowering. At least 100 f.c. of additional light are needed to induce flowering (table 2).

An experiment was carried out to determine the effectiveness of the different pairs of leaves in inducing flowering. Plants of *Baeria* were germinated, and from 5 days after germination on, kept in continuous light. When the cotyledons and two pairs of leaves were left, all

plants showed floral initiation 17 days after germination. When either the cotyledons or the first or second pairs of leaves were cut off but the others left (cotyledons and one pair of leaves or only two pairs of leaves), all plants showed flower primordia at 17 days. When only the cotyledons or the first or second pair of leaves were left on the plants, some had flowers initiated but others had not, and there seemed to be no difference between the groups. Apparently the cotyledons and leaves contribute equally to the change from vegetative to flowering in *Baeria*.

From the previous experiments it had become clear that plants of *Baeria* kept on short photoperiod did not flower, but that they could be used to test the effectiveness of various additional treatments in flower initiation. Since it was known that caulocaline and sugars could be absorbed by leaves submerged in solution (11), and since the linear leaves can easily be inserted in glass capillaries of solutions, experiments were run to study the effectiveness of pure substances and extracts on flower initiation. Plants were grown on 8-hour photoperiods, and as soon as either the cotyledons or first leaves were well developed they were inserted in capillaries thrust in the sand of the container. Each day the solution in the capillaries was renewed. None of the treatments resulted in initiation of flowers. The experiments are being continued. Substances tried were 3% and 6% sucrose, thiamin, pyridoxine, nicotinic acid, pantothenic acid, para-aminobenzoic acid, and biotin (all in 1 p.p.m.), and tomato and palm leaf extracts.

TABLE 3

LEAF DEVELOPMENT AND FLOWERING OF BAERIA PLANTS EXPOSED DAILY TO 8 HOURS OF WHITE LIGHT AND 16 HOURS OF COLORED LIGHT OF 250 F.C. (COLORS OBTAINED WITH CORNING GLASS FILTERS). TREATMENT WITH COLORED LIGHT FROM 5–13 DAYS AFTER GERMINATION; OBSERVATION AFTER 19 DAYS

Color of additional light	Approximate wave lengths	No. of leaves	Length (mm.)			Stage of flowering
			Cotyledons	First pair of leaves	Second pair of leaves	
Darkness	....	4	9.1	11.6	1.7	0.0
Red	5700–8000	6	8.3	20.6	17.6	3.2
Yellow	5000–8000	4	10.5	14.8	2.3	2.0
Green	4700–5800	3	9.0	12.1	1.0	0.0
Blue	3500–5300	4.6	9.6	17.3	13.7	1.0

Discussion

Baeria is a convenient plant for photoperiodic investigations, owing to its quantitative response, which makes it possible to express effects of light in terms of numerals. It can also be grown in low light intensities and responds quickly to treatment. The plants respond to a long photoperiodic treatment 5 days after planting, since two pairs of leaves, or the cotyledons and one pair of leaves, suffice for photoperiodic perception. As soon as cotyledons and the first pair of leaves are fully developed, the plant becomes sensitive to long photoperiods. In the case of millet (2) the plants become sensitive after 1 week, in soybeans (1) after 3 weeks.

Among photoperiodically sensitive plants there is a distinction between those in which there is a very narrow range in photoperiods giving either flowering or no flowering (as *Xanthium*, 3), or where there is a gradual change to flowering over a rather wide range of photoperiods (as soybeans, 1). *Baeria* belongs to this second group.

There also are differences in the intensity of supplementary light required to induce photoperiodic responses. In general, this intensity can be very low, mostly 10 f.c. or less (in aster even 0.3 f.c. is fully effective, 12). In *Baeria*, on the other hand, higher intensities are required of the same magnitude as necessary for photosynthesis. This may be the reason why the spectral sensitivity of *Baeria* differs so much from that of most other plants. Aster, stocks, soybeans, spinach, and many others (13) need red or yellow supplementary light to initiate flowers; blue light is completely ineffective. In *Baeria* only green light is ineffective, but both the long and the short wave lengths induce flowering. It would seem that photosynthesis beyond a 10-hour photoperiod required for vegetative development induces flower initiation, for the light intensity during the 10-hour short photoperiod can be varied within a very wide range (200–1500 f.c. or more in daylight) without resulting in flowering, whereas the supplementary light is effective whether during short photoperiods the plants were subjected to high or to low light intensity. For short-day plants photosynthesis is probably the controlling factor during the short photoperiod (7).

In *Baeria*, long photoperiod is effective only in inducing flowers when the plants are kept below 25° C. Such a relation between photoperiodic behavior and temperature has been known to exist in many plants (9, 8). According to ROBERTS and STRUCKMEYER, alfalfa, pea, nasturtium, primrose, and red clover are all examples of long-day plants which do not flower when kept on long warm days. But *Baeria* differs very much from such long-day plants as sugar beet and *Rudbeckia*, in which low or high temperatures may substitute for long photoperiods (6, 5).

HAMNER and BONNER (3) tried the application of many extracts or pure substances to *Xanthium* kept on long photoperiods, but in no case did they induce flowering. The present experiments with applied substances were equally unsuccessful.

Summary

1. *Baeria chrysostoma* is a long-day plant, provided it is grown below 25° C. It will grow at light intensities of above 150 f.c., obtained with fluorescent tubes, and responds rapidly to long photoperiods.

2. At least 5 long photoperiods are required for flower initiation, and a 15-hour photoperiod is effective. Plants become sensitive to long photoperiods 5 days after germination; and a few days after the long photoperiodic treatment, microscopic observation shows flower initiation.

3. The intensity of the supplementary light required after an 8-hour photoperiod for flower initiation is above 100 f.c. Both the short and the long wave lengths are effective, but green light is not. The cotyledons and leaves are equally effective in perception of the light effect.

4. No extracts or pure substances induced flowering in plants kept on short photoperiods.

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LITERATURE CITED

1. BORTHWICK, H. A., and PARKER, M. W., Photoperiodic perception in Biloxi soybeans. BOT. GAZ. 100:374–387. 1939.
2. CAJLACHJAN, M. C., The age of plants and the photoperiodic reaction. Compt. Rend. Acad. Sci. U.R.S.S. 6:306–314. 1933.
3. HAMNER, K. C., and BONNER, JAMES, Photoperiodism in relation to hormones as factors in floral initiation and development. BOT. GAZ. 100:388–431. 1939.
4. MUNZ, A. P., A manual of Southern California botany. Claremont, California. 1935.
5. MURNEEK, A. E., Length of day and temperature effects in *Rudbeckia*. BOT. GAZ. 102:269–279. 1941.
6. OWEN, F. V., CARSNER, E., and STOUT, M., Photothermal induction in sugar beets. Jour. Agr. Res. 61:101–124. 1940.
7. PARKER, M. W., and BORTHWICK, H. A., Floral initiation in Biloxi soybeans as influenced by photosynthetic activity during the induction period. BOT. GAZ. 102:256–268. 1941.
8. ROBERTS, R. H., and STRUCKMEYER, B. E., Further studies of the effects of temperature and other environmental factors upon the photoperiodic responses of plants. Jour. Agr. Res. 59:699–710. 1939.
9. THOMPSON, H. C., Temperature as a factor affecting flowering of plants. Proc. Amer. Soc. Hort. Sci. 30:440–446. 1934.
10. WENT, F. W., Plant growth under controlled conditions. I. The air-conditioned greenhouses at the California Institute of Technology. Amer. Jour. Bot. 30:157–163. 1943.
11. WENT, F. W., and BONNER, D. M., Growth factors controlling tomato stem growth in darkness. Arch. Biochem. 1:439–452. 1943.
12. WITHROW, R. B., and BENEDICT, H. M., Photoperiodic responses of certain greenhouse annuals as influenced by intensity and wavelength of artificial light used to lengthen the daylight period. Plant Physiol. 11:225–249. 1936.
13. WITHROW, R. B., and WITHROW, A. P., Effect of various wavebands of supplementary radiation on the photoperiodic response of certain plants. Plant Physiol. 15:609–624. 1940.

CYTOLOGY AND MICROCHEMISTRY OF NUCLEI IN DEVELOPING SEED OF ECHINOCYSTIS MACROCARPA

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Introduction

Nuclei of relatively enormous size have been described in the differentiating vessels of *Ricinus communis* and of *Echinocystis macrocarpa* (12, 13). In the developing seeds of *Echinocystis*, even larger nuclei appear and are visible to the naked eye.

In reference to rapidly segmenting animal eggs, PAINTER asks, "How is it possible for the chromosomes in rapidly segmenting eggs to synthesize so quickly the new material needed in the reduplication process prior to each cell division?" It is seen that in *Drosophila* the nurse-cell nuclei increase about 512 times in volume. Cells and nuclei are later absorbed by the developing egg. From further evidence such as this PAINTER concludes that "the rapid building of the cleavage chromosomes is possible in the segmenting egg because the synthesis is more in the nature of a reassembling of already existing materials such as nucleotids, etc. under the guidance of active chromosomes rather than an actual synthesis of building blocks from relatively simple substances" (10).

In botanical terms this question may be restated as follows: How is it possible for the chromosomes to synthesize the materials necessary for the innumerable mitoses which result in the dense small-celled tissue of the rapidly developing embryo cotyledons? In the plant, nucellus and endosperm replace the nurse cells and presumably function

as the immediate source of the building materials necessary for nuclear and cytoplasmic protein synthesis.

The present paper serves as an introduction to the cytology and microchemistry of the giant nuclei present in the nucellus and endosperm of the developing seed.

MATERIAL AND METHODS.—In southern California, *Echinocystis macrocarpa* flowers more or less continuously from January until about the end of April. Fresh material in all stages of growth is therefore available throughout this period. The inferior 4–6 locular ovary measures 0.3–0.5 cm. in length. Locules may contain 4–6 seeds, some of which may fail to develop. The mature spiny fruit measures 7–10 cm. in length.

Undoubtedly the most significant recent tool in cytological investigation is the ultraviolet technique of CASPERSSON (1). In the wartime absence of essential equipment, microchemical tests must necessarily suffice in a survey of the distribution of chromatin and other ergastic substances. The following methods or reagents were used: (*a*) Feulgen's reaction–nucleic acid (6, 8, 9); (*b*) ninhydrin, triketohydrindene hydrate–amino acids (7); (*c*) Millon's reagent–tyrosin; (*d*) trypsin, with or without the addition of lanthanum chloride–protein digestion (1); (*e*) Fehling's solution–reducing substances (15); (*f*) Sudan III–fats; (*g*) iodine potassium iodide; and (*h*) ferric chloride–starch and tannin, respectively. The amount of free water present in the nucleus was determined as before by dehydration in absolute alcohol (12, 13).

In preliminary observations on pH, the range indicator method was used (14). In addition to the standard series of dyes, thionin (Lauth's violet) also proved of interest (3). Permanent preparations were stained with haematoxylin, with or without such counterstains as erythrosin and orange G. So far no fixative has been found which prevents considerable shrinkage.

Observations

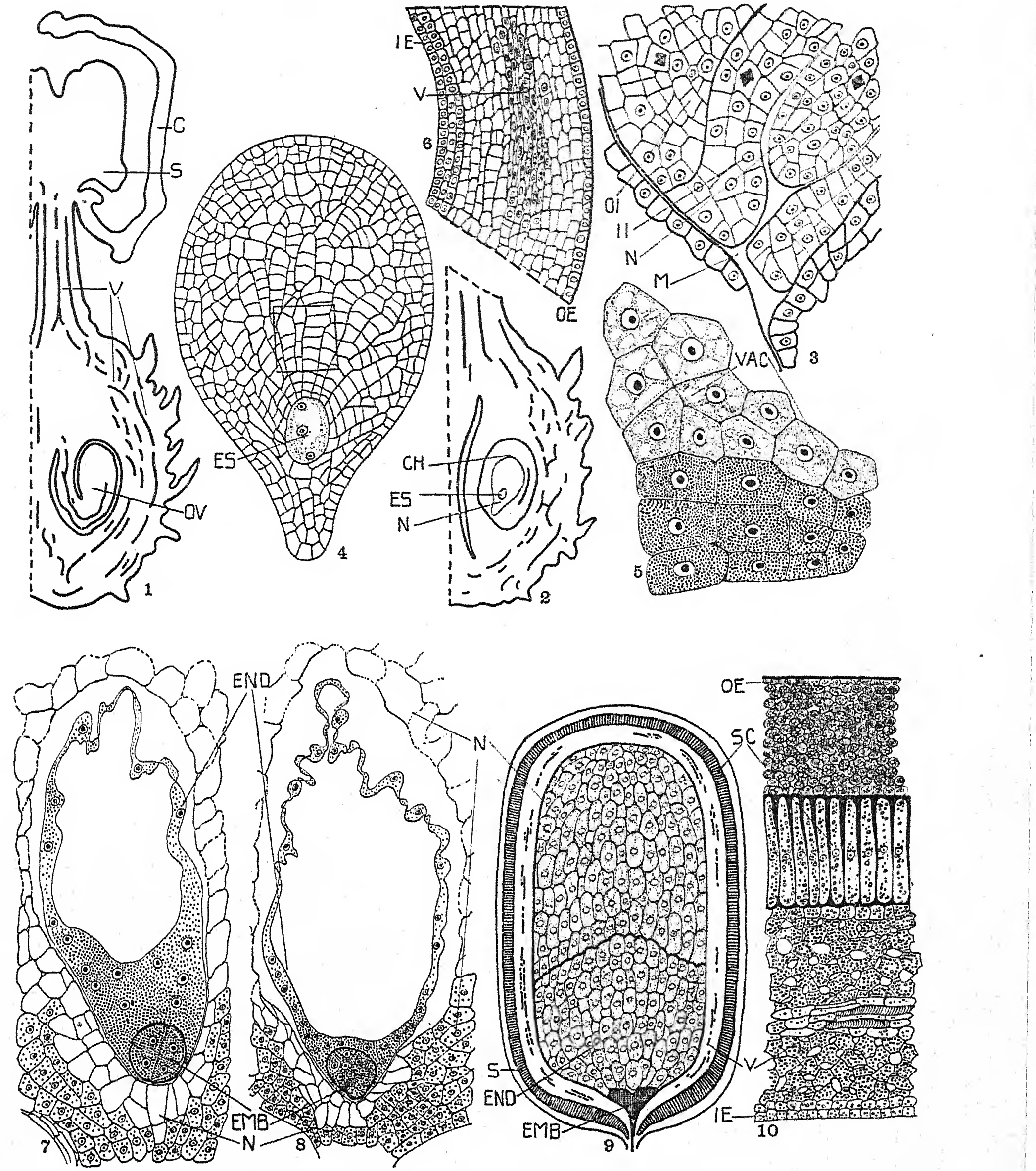
MORPHOLOGY OF SEED DEVELOPMENT

Differentiation of the megagametophyte, fertilization, and subsequent growth of the seed follow the general pattern described by KIRKWOOD (4) and KRATZER (5) for the Cucurbitaceae as a whole. A brief summary will therefore suffice at this time.

The megagametophyte when mature is deeply imbedded in the nucellus of the anatropous ovule. Two integuments are present—the inner slight, only three cells thick; the outer complex and many-layered. In the latter a differentiating vascular network is already apparent. The main axis of this reticulum encircles the seed and is continuous with the funicular vascular strand. In the mature seed it underlies the definitive dark ridge of the seed coat. The nucellus extends into the micropyle as a nucellar neck. The ovule at this stage measures 0.5 mm. in length (figs. 1–6).

After fertilization, growth of seed and fruit is rapid, but the embryo remains practically invisible to the naked eye until the seed is more than half-grown. The endosperm is at first coenocytic as usual. Thereafter cell formation begins in the embryonic zone, while the distal endosperm penetrates the nucellus as a noncellular coecum (figs. 7–11).

Mature seeds measure ± 2 cm. in length. When the seeds first reach maximum volume, the seed coat is white, soft, and unlignified, but is highly differentiated as to outer integument. Starch is abundant in all except the palisade cells.



FIGS. 1–10.—Diagrammatic drawings: Fig. 1, unopened flower, longisection showing attachment and vascular supply of one ovule. Fig. 2, at another level; position of megagametophyte evident. Fig. 3, same; inner and outer integuments surround micropyle and nucellar neck; mitosis throughout. Fig. 4, same, nucellus and megagametophyte; cells of nucellus immediately adjacent to latter are densely filled with protoplasm, while remainder are already vacuolated. Fig. 5, vacuolating and nonvacuolated cells of nucellus. Fig. 6, testa, longisection; developing vascular strands evident, otherwise seed-coat tissues are relatively undifferentiated; multinucleolate nuclei appear in vascular tissue. Fig. 7, seed, longisection (0.5 mm.); embryo in 8-cell stage with surrounding endosperm. Fig. 8, seed, longisection (2.3 mm.); multicellular embryo. Fig. 9, diagrammatic surface view of seed after partial removal of testa (2 cm.); endosperm, now cellular, occupies nearly half seed cavity; dark line indicates limit of nucellus identifiable on dissection; testa shows micropyle, differentiating sclerenchyma, and vascular tissue. Fig. 10, testa, longisection; sclerenchyma vascular tissue, starch-filled ground cells and intercellular spaces evident. *c*, corolla; *ch*, chalaza; *emb*, embryo; *en*, endosperm; *es*, megagametophyte; *ie*, inner epidermis of testa; *ii*, inner integument of ovule; *m*, micropyle; *n*, nucellus; *nc*, nucleus; *ncl*, nucleolus; *oe*, outer epidermis of testa; *oi*, outer integument of ovule; *ov*, ovule; *s*, stigma; *sc*, sclerenchyma of testa (differentiating); *v*, vascular and provascular tissues;

The seed cavity is filled with a firm gelatinous transparent ellipsoidal mass, nucellus and endosperm. The surface cells are small, but the inner cells and nuclei are visible to the naked eye. The endosperm gradually encroaches upon and absorbs the nucellus. Morphologically these tissues are indistinguishable in surface view, but when separation is effected by careful probing the smaller cells of the endosperm surface layer are apparent (figs. 9–11). All cells are highly turgid, and when punctured the contents spurt out forcibly in all directions. When the cells are so punctured the nuclei are set free and float in the cytoplasmic fluid. No mounting fluid is required, and this material is therefore excellent for nuclear investigation.

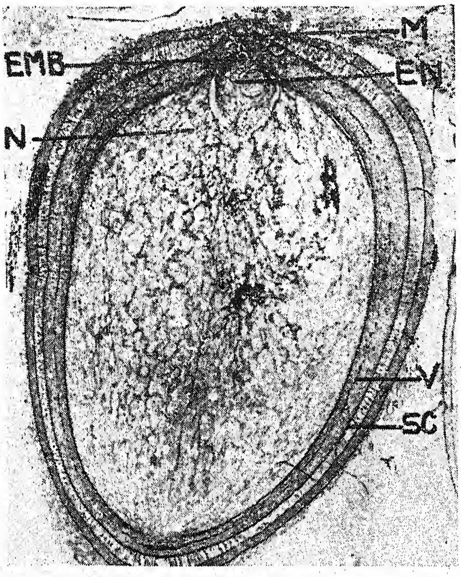


FIG. 11.—Seed, diagrammatic longisection (1.3 cm. after imbedding); embryo, cellular endosperm, region of coecum penetration indicated by partial breakdown of surrounding nucellus, and intact cellular nucellus occupy seed cavity.

Hardening of the seed coat begins with the lignification, near the micropyle, of the palisade layer as it develops into sclerenchyma tissue. Within the seed cavity, while the nucellus is being gradually resorbed by the endosperm, both are in turn being consumed by the now rapidly expanding embryo. During this resorption the cells lose their turgor, the cell walls gradually break down, the nuclei shrink in volume, and the whole tissue disintegrates in a semifluid, somewhat viscous mass and finally disappears. The embryo cotyledons occupy the entire seed cavity. Tannin-like substances darken the ripe seed coat.

The comparative sizes of fruit, seed, and embryo, and also of cell and nuclear size in endosperm and nucellus, are seen in the typical measurements given in table 1. The visibility of cells and nuclei is readily understood on examination of their dimensions in the full-grown seeds.

NUCLEAR MORPHOLOGY

When the megagametophyte is mature, the cells of the nucellus are already varied in size and in extent of vacuolation. Surface cells, the cells of the neck and those immediately around the embryo, are smaller and contain relatively dense protoplasm. The larger central cells are vacuolated. Cell division is active throughout and all stages of mitosis are seen (figs. 4, 5).

Cell division is practically complete about the time of fertilization, and increase in nucellar volume results from cell expansion. This is clear from the cell count along the major and minor axes of the elliptical nucellus as seen in longitudinal section (fig. 4). Thus at this early stage the number of cells along the longitudinal and transverse axes is $\pm$ 19 and $\pm$ 14, respectively. In a typical seed practically full-grown, the cells across the latter, the minor axis, number $\pm$ 14. In the meantime, in the seed cavity endosperm has replaced approxi-

mately one-half the nucellus. Along the remaining major radius of the nucellus the cells now number ± 9 (fig. 9). The role of cell expansion is thus clearly indicated. In the endosperm cell division is completed soon after fertilization, and since the endosperm cells parallel the nucellar in volume increase, the nutritive tissue—during development—superficially appears homogeneous.

In the young nucellus about the time of fertilization, the nuclei of all cells are relatively uniform, and it is possible to describe a "typical" nucleus. Such a body measures 7–10 μ in diameter. Except during actual division, the chromatin appears in the interkinetic "resting" condition. One nucleolus is present as a rule, but two or occasionally three may be observed (fig. 5). In specialized cells only, such as the differentiating vascular elements of the outer integument, nucleoli are more frequent and range 4–7 in number (fig. 6).

In older seeds, endosperm and nucellar nuclei so far remain morphologically and microchemically indistinguishable, but it is no longer possible to talk of a typical nucleus. While superficially the chromatin network appears similar throughout, the nucleolar condition is chaotically variable. The chromatin consists of strands which vary in thickness from 0.5 to 3 μ. The latter may be more or less clearly observed in all types of preparations: in living nuclei before or after vital staining, in transmitted light or in dark field, in preparations stained with acetocarmine or with microchemical reagents such as Feulgen or ninhydrin, and in permanent haematoxylin-stained mounts. The orientation of the major strands and of the larger nucleoli is interdependent. The thicker chromatin threads invariably appear to radiate from the latter. The lesser nucleoli also occur at chromatin-strand intersections (figs. 12–16).

The finer chromatin strands present the usual "beaded" appearance. The thicker vary erratically in width throughout their length and at intervals may be clearly resolved into a number (generally 3–5) of finer component beaded threads. The theoretical concept of the multiple structure of the salivary chromosomes of *Drosophila* is thereby suggested. In this phase of nuclear development, however, structural details in the reticulum cannot be definitely resolved by ordinary optical means. It appears probable that increase in chromatin depends on the multiplication of the chromonemata and occurs during cell and nuclear expansion. The conditions which favor volume increase are therefore identical with, or accompany, those which inhibit the completion of mitosis.

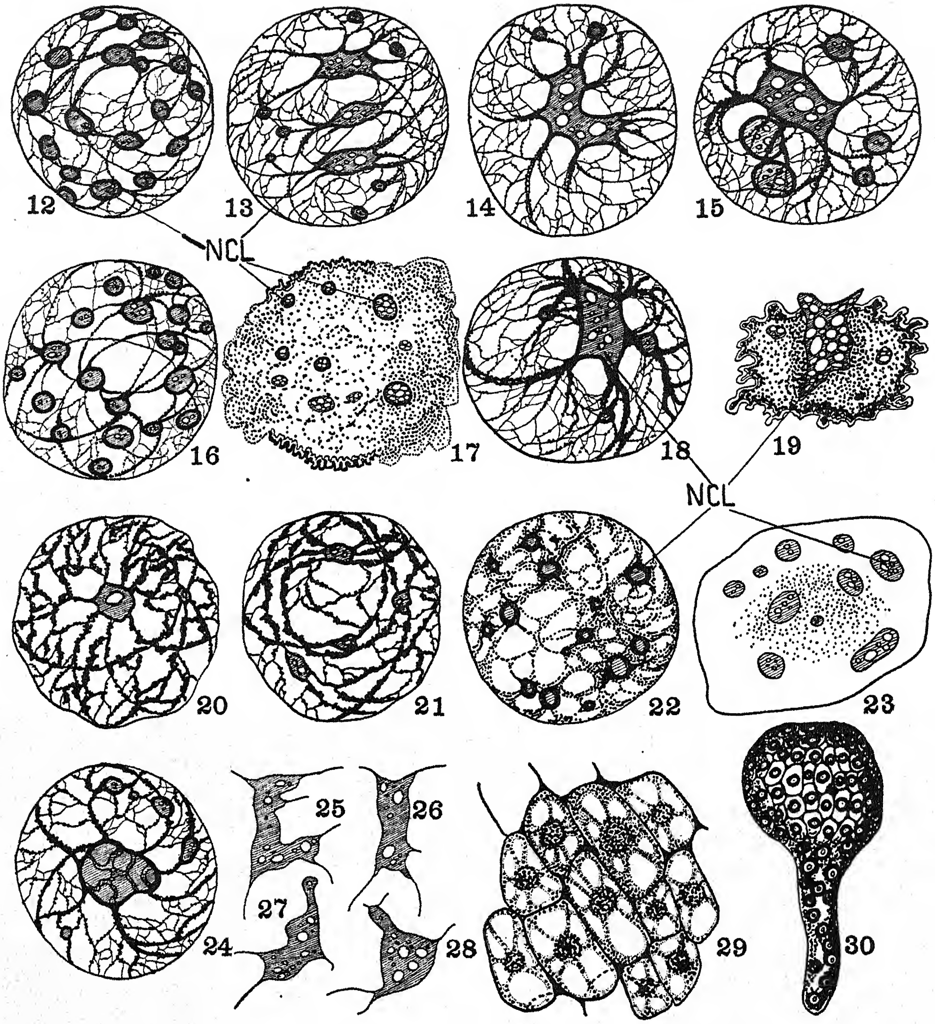
TABLE 1

MEASUREMENTS OF FRUITS OF VARYING SIZE AND TYPICAL INCLOSED SEED EMBRYOS, CELLS, AND NUCLEI

Fruit length (cm.)	Seed length (cm.)	Cotyledon length (cm.)	Cell size* (μ)	Diameter of largest nuclei (μ)
1.4	0.2	Microscopic	76	15
2	0.6	Microscopic	256	40
3.2	1	Microscopic	350	64
4.2	1.4	0.05	660	....
5.8	1.7	....	....	104
7.5	2	0.5	800	114
8	1.9	0.5	800	123
8	2.0	....	2800	200

* Majority are ellipsoidal; mean of major or minor axes recorded here.

The nucleoli vary in size, in consistency, and in outline. Diameters vary from 0.2 to 40 μ, and may therefore be only roughly graded as large, inter-



FIGS. 12–30.—Figs. 12–15, nuclei (diameters 152, 104, 95, 96 μ); nucleoli varied in size and number. Figs. 16, 18, nuclei (190 and 120 μ) before digestion. Figs. 17, 19, same during digestion in trypsin. Figs. 20, 21, after treatment with trypsin and lanthanum chloride and subsequent staining in Feulgen; nuclear structure persists. Fig. 22, nucleus (diameter 95 μ); after treatment with Millon. Fig. 23, nucleus (major and minor axes, 152 and 95 μ) during treatment with Fehling's. Fig. 24, nucleus (diameter 57 μ); nucleolus fragmented. Figs. 25–28, nucleoli of varying outlines. Fig. 29, nucellar cells after treatment with Fehling's; density of cuprous-oxide precipitate indicated by black granules. Fig. 30, differentiating endosperm dissected out; 15 free nuclei in noncellular coecum (4800 μ). Semidiagrammatic drawings of nuclei (figs. 12–28) based on camera-lucida outlines.

mediate, and small. Values such as 30 μ, 15 μ, and 5 μ may be arbitrarily assigned to these terms in order to clarify general morphological description. Nucleolar composition is heterogeneous. Vacuoles of undetermined content and of all sizes are scattered throughout. In smaller nucleoli the conventional spherical or elliptical outline is generally maintained, but in the larger ones fantastic forms occur. Such outlines presumably result from internal changes, accompanied by alterations in surface tension. After a survey of some 250 living nuclei, and later intensive study of permanent preparations, only a few generalizations appear valid in relation to nucleolar number, outline, and distribution:

1. In nuclei 10–20 μ or less in diameter, only one nucleolus is present as a rule, but occasionally two may appear (fig. 5).

2. When nuclear diameter ranges 25–40 μ, the nucleoli may number 1–4. Exceptions occur, however, and 5–7 nucleoli are sometimes observed.

3. Conditions in the larger nuclei, 40–200 μ, are chaotic. No correlation is at present evident between nuclear volume and nucleolar number. Various patterns are observed: (*a*) All nucleoli are comparatively small, and their number appears indefinite (fig. 12). They constitute the entire nucleolar material. (*b*) Nucleoli of intermediate size and relatively limited number (fig. 13) are dominant. They are almost invariably accompanied by one or several smaller units. (*c*) The number of large nucleoli is necessarily spatially limited. One to three may occur, but, as before, one or more of the smaller elements is usually present. The outline may be conventional, spherical, elliptical, or entirely erratic (figs. 14, 15, 25–28). (*d*) Some of the largest nucleoli appear fragmented. The nucleolar membrane remains intact, but the body is subdivided into irregular segments, as if ready to split apart. The significance of this condition is not clear. Present evidence suggests that the nuclei increase in number gradually, not suddenly, as would be the result of sudden fragmentation (fig. 24).

Since nucleolar number is in general considered an index of chromosome duplication, the conclusion is inevitable that, in the largest multinucleolate nuclei, actual polyploidy lies beyond the range of microscopic definition by means of visible light only. Ultraviolet technique may contribute to the detailed analysis of this complexity of structure.

Nuclear microchemistry

The question of the source of nucleoproteins for the embryo in its phase of rapid growth has already been stated. The full answer will necessarily depend on accurate quantitative analysis by chemical methods, together with ultraviolet techniques. Routine microchemical tests, however, are of use in a general survey.

Feulgen's reagent.—Mild hydrolysis of nucleoprotein followed by the Schiff reaction of the resulting aldehydes produces more or less intense purple coloration throughout the cell. The chromatin network is deeply stained, and the color of the nucleoli is almost equally intense. The karyolymph is lighter in color. Beyond the limits of the nucleus, cytoplasm and cytoplasmic granules, grading down to the limits of visibility, are also distinctly stained. As already demonstrated by Caspersson and Schultz (2), nucleic acid is present in structures other than chromatin.

During nucellar and endosperm resorption, as the embryo develops these disintegrating tissues stain even more

heavily. Remains of shrunken nuclei may still be recognized. Granules are abundant in nuclei and in cytoplasm. Intensity of color depends in part on the concentration of material resulting from loss of water.

During the period of active growth of the embryo, the nutritive tissues, nucellus and endosperm, thus furnish a volume of available nucleoproteins. To what degree these building materials are broken down prior to absorption by the embryonic tissues remains to be determined. Detailed quantitative analysis would yield useful information regarding the amount of material used in embryonic growth compared with the supply immediately available in endosperm and nucellus.

Ninhydrin.—All naturally occurring amino acids give an intense blue coloration on addition of ninhydrin (7), so that this reaction is in no wise specific for any particular amino acid. As expected, at all stages of growth the chromatin, nucleoli, and karyolymph of the nucleus —and also the abundant granules and matrix of the cytoplasm—become, on mild warming (50°–60° C.), more or less deeply stained. Ninhydrin functions almost as well as any chromatin stain in resolving details of the reticulum.

Millon's reagent.—The presence of the tyrosin complex was noted in the tuber of *Echinocystis* (13). Treatment of the developing seeds with Millon's reagent produces an equally positive reaction. Nucellus, endosperm, and embryo are immediately stained more or less deeply reddish brown. Tissue shrinkage occurs and the darkening color completely masks details of structure in the cell as a whole. The free nuclei, however, which are present in any preparation, are readily observed. Loss of nuclear volume is evident on entrance of the reagent, and the characteristic reddish color appears almost immediately. The nucleoli, if irregular in outline, round off and become spherical. In all, the vacuoles merge in the center, and the surrounding reddened crater-like rim of nucleolar matrix remains refringent (fig. 23). Chromatin becomes granular and stains more heavily than karyolymph. The occurrence of this amino acid in nuclei is not unusual.

Fehling's solution.—Reducing sugars are commonly distributed in nutritive as in other tissues. In nucellus and endosperm the reaction to Fehling's solution is readily seen microscopically. On careful and continued addition of boiling KOH (15), the cytoplasm and nuclei rapidly change from blue to mustard yellow, to orange, and eventually to deep red-brown. In the cytoplasm, as the color deepens, cuprous oxide precipitate appears around the periphery of the cell, along the radiating cytoplasmic strands, on the surfaces of the vacuoles, and—most conspicuously—as a corona masking the nucleus (fig. 29). In the nucleus itself color change parallels that in the cytoplasm. Details of chromatin outline are soon lost, but nucleoli remain distinct and are equally stained (fig. 23). The nucleus continues to darken, and further observation is effectively blocked by the intensity of the internal precipitate and by the cytoplasmic corona already noted.

This reaction denotes reducing substances within the nucleus itself. Whether these exist free within the karyolymph, or result from hydrolysis of nuclear components during the reaction, is undetermined. Absorption of water might be attributed entirely to adsorption by nucleoproteins, or it may be linked with osmotic substances. In analyses of sperm and similar nuclei so far available,

monosaccharides and similar substances do not appear. Detailed analyses of hypertrophied nuclei of high water content, such as the present, are lacking, however. The identity of these Fehling's reducing substances therefore remains to be determined.

TRYPSIN.—Microchemical dissection by means of enzymes affords further evidence regarding nucleic-acid distribution (2). In qualitative experiments, digestion by powdered trypsin was followed by the Feulgen test. The process of digestion may be observed microscopically and is complete in 15–20 minutes or less. The nucleus shrinks markedly in volume within 3–5 minutes, but details of nucleoli and nuclear network are not thereby obscured. The nuclear membrane appears slightly thicker and thereafter becomes more or less finely puckered. The nucleoli also shrink and very soon increase in vacuolation, but the spongy matrix which results remains visible and highly refringent (figs. 16–19). The nuclear membrane now thins out along one or more sectors and eventually breaks. In the meantime the entire nuclear material has become finely granular. The granules, $\pm$ 1 μ and grading down to the limits of visibility, are in active Brownian movement and escape on rupture of the nuclear membrane. The enzyme trypsin, by digestion of protein, thus disrupts completely the continuity of the chromatin mesh. The slightest pressure on the coverglass is sufficient to disperse the particles and to remove completely all traces of nuclear outline.

This disintegration may be effectively blocked by addition of lanthanum chloride (figs. 20, 21). Nucleic acid, when freed from the nucleoprotein complex during trypsin digestion, immediately reacts with lanthanum ion and precipitates. The continuity of the chromatin complex remains unimpaired. The contrast between fragmented and intact nuclei is excellently seen on careful addition of Feulgen's reagent.

WATER CONTENT.—To determine the volume of removable water present, the technique of dehydration in absolute alcohol is repeated here (12). The results are given in table 2. As in other large nuclei examined, this volume ranges 60–80%. The actual increase in nuclear structural materials, chromatin, etc., is estimated by comparison of the volumes of a fully contracted giant nucleus and a typical meristem nucleus. In one case (original diameter 104 μ) the volume after dehydration is 117,424 cu. μ, while the volume of a typical meristem nucleus is $\pm$ 33 μ^3. Growth in structural material is therefore approximately 3558-fold. The significance of this increase in nucleoproteins in relation to embryonic growth and food supply has already been mentioned.

TABLE 2

PERCENTAGE VOLUME OF WATER IN NUCLEI INDICATED ON CONTRACTION IN ABSOLUTE ALCOHOL

Initial diameter (μ)	Volume (%)		
	Initial (μ^3)	After contraction (μ^3)	Loss
43	43,601	14,801	63
57	96,762	20,900	78
96	462,274	12,816	72
108	658,199	62,980	81

pH.—Protein synthesis is conditioned by pH, in particular by the isoelectric point of the dominant protein, among other factors (11). In meristematic tissues, including the cambium, mitosis alternates regularly with nucleoprotein synthesis (nuclear growth). In endo-

sperm and nucellar tissues, nuclear expansion occurs, but nuclear division fails to follow. Comparison of pH in these contrasting tissues is therefore of interest. Toward the end of the growing season, when pH determination by means of the range indicator method was attempted but while seed was still available, good stem material was already scarce. The pH of the nuclei of the cambial cells, ± 4.6, is markedly contrasted with that of the endosperm and nucellus, ± 6. These results are tentative only. Should further investigation confirm these divergent values, then pH may prove one significant factor in the fundamental processes of nuclear growth and chromosome division.

Preliminary tests with the oxidation-reduction indicator thionin (3) showed rapid penetration and at the time of entry differentiation of nucleoli, chromatin, and karyolymph. The first two stain clearly blue, as does lignin in a stem section; the latter light purple, as does cortical or pith parenchyma. This difference is later masked, and the nucleus as a whole appears purple. So far, however, it is the only indicator in the series used which distinguishes clearly between the component structures of a living nucleus.

Summary

1. Development of the seed in *Echinocystis macrocarpa* follows the general outline given by KIRKWOOD for the Cucurbitaceae as a whole.

2. The cells and nuclei of endosperm and nucellus are visible to the naked eye in the full-grown but still unripened seed. The largest nuclei measure 150–200 μ in diameter. The nucleoli are extremely variable in number and outline.

3. The role of protein in maintaining the continuity of the chromatin strands is confirmed by qualitative experiments with the digestive enzyme trypsin. Disintegration of the nuclear reticulum by trypsin is inhibited by lanthanum chloride.

4. The reaction of Fehling's solution indicates the presence of reducing substances within the nucleus itself.

5. Actual increase in nuclear structural materials may be 3558-fold.

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LITERATURE CITED

1. CASPERSSON, T., Über den chemischen Aufbau der Strukturen des Zellkernes. Skand. Arch. Physiol. 73. Suppl. 8. 1936.
2. CASPERSSON, T., and SCHULTZ, J., Ribonucleic acids in both nucleus and cytoplasm and the function of the nucleolus. Proc. Nat. Acad. Sci. 26:507–514. 1940.
3. CLARK, W. M., Studies on oxidation-reduction. Hygien. Lab. Bull. 151. 1928.
4. KIRKWOOD, J. E., Comparative embryology of the Cucurbitaceae. New York Bot. Gard. Bull. 3:313–402. 1903–5.
5. KRATZER, J., Verwandschaftlichen Beziehungen der Cucurbitaceen auf Grund ihrer Samenentwicklung. Flora 109–110:275–343. 1916–1918.
6. LEE, B. (edit: GATENBY), Microtomist's Vade-mecum. Philadelphia. 1928.
7. LLOYD, D. J., and SHORE, A., Chemistry of proteins. Philadelphia. 1938.
8. MAZIA, D., Enzyme studies on chromosomes. Cold Spring Harbor Symposia on Quantitative Biology 9:40–46. 1941.
9. METZ, C. W., Structure of salivary gland chromosomes. Cold Spring Harbor Symposia on Quantitative Biology 9:23–39. 1941.
10. PAINTER, T. S., On the synthesis of cleavage chromosomes. Proc. Nat. Acad. Sci. 26:95–100. 1940.
11. PEARSALL, W. H., and PRIESTLEY, J. H., Meristematic tissue and protein isoelectric points. New Phytol. 22:185–191. 1923.
12. SCOTT, FLORA M., Size of nuclei in the shoot of *Ricinus communis*. BOT. GAZ. 101:625–636. 1940.
13. ———, Survey of anatomy, ergastic substances, and nuclear size in *Echinocystis macrocarpa* and *Cucurbita pepo*. BOT. GAZ. 104:394–408. 1943.
14. SMALL, J., Hydrogen ion concentration in plant cells and tissues. Berlin. 1929.
15. TUNMANN, O., and ROSENTHALER, L., Pflanzenmikrochemie. Berlin. 1931.

CYTOLOGICAL AND TAXONOMIC STUDIES IN THE GENUS BRODIAEA. II

MADELINE PALMER BURBANCK

Introduction

In a previous paper (1), the chromosome numbers of twenty-four species and varieties of *Brodiaea* were reported, and taxonomic and cytological findings seemed to justify the separation of the plants into three genera—*Brodiaea*, *Dichelostemma*, and *Triteleia*. In some instances data on the plants were incomplete. Further observations have now been made, certain specific delimitations examined in more detail, and two additional species have been investigated and their chromosome numbers determined.

The bibliography on *Brodiaea* as given previously (1) has since been found to be incomplete. Following the classification of the monocotyledons as given by HUTCHINSON (6), FLORY and YARNELL (2) list chromosome numbers for *Brodiaea* and *Brevoortia* (*Dichelostemma*) under the Amaryllidales. The numbers given and the investigators cited are essentially the same as those already reviewed by the writer, with one addition. The diploid chromosome numbers of 12 for *Brodiaea uniflora* and 36 for *B. grandiflora* are listed, with YAMAMOTO given as the investigator. TISCHLER (7) also includes these species in his summary of *Brodiaea* chromosome numbers and gives the haploid numbers of 6 and 18, respectively, with YAMAMOTO as the authority for the counts. HOOVER (3) does not include *B. uniflora* and other South American "Brodiaeas" in his classification of North American Brodiaeas, and the species has not been investigated in connection with these studies. The number 36 for *B. grandiflora* does not agree with other reports on the species. Of the three species to which the name "*grandiflora*" has been applied, two have the diploid chromosome number of 32 and the third, 42 (1).

Material and methods

The plants of *Brodiaea* reported upon earlier have been under observation for two additional years. In the fall of 1941 the majority of the corms were planted in the ground or in pots sunk in the ground in Moorestown, New Jersey, where they bloomed the following spring. Corms of *Dichelostemma volubile* and *D. ida-maia*, the hardiness of which was doubtful in this climate, and corms of *Triteleia laxa*, *T. laxa* Blue King, and *T. candida*, were taken to New York City and grown in the greenhouse on the top floor of Schermerhorn Extension, Columbia University. Also grown in the greenhouse were additional corms of *T. laxa*, *T. laxa* Blue King, and *D. ida-maia* purchased from Mr. CARL PURDY, Ukiah, California, and corms of *T. laxa* and *Bloomeria crocea* kindly sent by Dr. R. F. HOOVER of Yakima, Washington. Studies of the somatic and meiotic chromosomes of the greenhouse plants were made in the usual manner by root-tip sections and acetocarmine smears of anthers. Root tips were fixed in either La Cour's 2 BE or Craf's solution. Slides made from the latter material tended to have clearer cytoplasm, but the actual outlines of the chromosomes were more definite in the material fixed in the 2 BE.[1]

[1] I wish to thank DR. S. F. TRELEASE and DR. M. M. RHOADES of the Botany Department of Columbia University for allowing me to use greenhouse space and laboratory facilities to carry on this investigation.

Late in the summer of 1942, when the corms were dormant, both the greenhouse plants and those grown outdoors in New Jersey were taken from the ground, sacked, and replanted in pots or in open ground outdoors in Springfield, Missouri. All species and varieties survived the winter of 1942–43, although some thrived better than others. No cytological studies were made of the plants while growing in Missouri, but records were kept of vegetative and flower characters.

Drawings were made at table level with the aid of a camera lucida. The magnification is approximately 1660. Drawings of mitotic chromosomes are from sectioned root-tip material and meiotic figures are from aceto-carmine smears of anthers.

Further observations on Brodiaea Dichelostemma, and Triteleia

Supplementary data have been collected on the plant material analyzed in detail earlier (1). Within the genus *Brodiaea* in the limited sense, *B. purdyi* and *B. minor* continued to exhibit marked size differences when grown under natural conditions. The longer scapes and larger flowers of *B. purdyi* were very apparent. The color forms of *B. californica* were distinguishable in New Jersey, where the corms were kept separate. They were not kept separate in Missouri, and when in bloom, the flowers all appeared to be the same color.

The material received from PURDY under the names *Brodiaea capitata* and *B. capitata* var. *multiflora* was seen in bloom in 1942 and 1943. The violet flowers corresponded to the description of *Dichelostemma pulchellum* (the species determination given earlier based on synonomy), with one exception. Six anthers were not always present, the three smaller stamens opposite the outer segments being sometimes absent or only one or two present. There were no morphological differences between the flowers of the species and of the "variety," but the variety exhibited a slightly greater profuseness of bloom. Since the chromosome numbers are the same, the plants received as var. *multiflora* are probably merely a more vigorous form of *D. pulchellum*.

Previously it was noted (1) that the chromosomes of the species of *Dichelostemma* examined were characterized by smaller diameters than those of the *Triteleia* and *Brodiaea* chromosomes. Dr. WODEHOUSE examined the pollen of *Dichelostemma volubilis* and found the grains to be only about half the size of the pollen grains of *Triteleia* (*Brodiaea*) *laxa*, and with an entirely different pattern of the exine (private correspondence). This is further justification for division of the large group, *Brodiaea*, into separate units.

The corms of both *Brodiaea peduncularis* and *B. eastwoodii*, grouped together by HOOVER (5) into the one species, *Triteleia peduncularis*, bloomed in Missouri. Although only three flowering scapes were produced by *B. eastwoodii* and two by *B. peduncularis*, table 1 shows that *B. peduncularis* produced consistently longer pedicels, as maintained by PURDY. The very short pedicel, 2.1 cm., produced by one scape of *B. peduncularis* was an exception, as can be seen by the averages. The flowers of the two plants were slightly different, although both fitted the description of *T. peduncularis* as given by HOOVER (5). The scapes of *B. peduncularis* were more slender than those of *B. eastwoodii*. The perianths of *B. peduncularis* were white, each segment with a bluish gray-green line down the

back which gave a bluish tinge to the inner surface; the anthers were white or sometimes bluish and somewhat reduced in size. The perianths of *B. eastwoodii* were creamy white on the inner surface, with a green line down the back of each segment; the anthers were white. On the basis of these observations, the chromosome numbers of 2n = 14 for *B. eastwoodii* and 2n = 28 for *B. peduncularis*, and the discussion presented earlier (1),

TABLE 1

COMPARISON OF B. EASTWOODII AND B. PEDUNCULARIS

Species	No. of flowers	Length of scape (cm.)	Pedicel length (cm.) Average	Pedicel length (cm.) Range
		Scape I		
B. eastwoodii	17	39.0	3.62*	2.2–4.4
B. peduncularis	5	19.5	4.88†	2.1–6.2
		Scape II		
B. eastwoodii	11	31.5	3.06*	1.5–4.7
B. peduncularis	7	20.0	5.97†	3.2–7.8

* Average 3.34. † Average 5.43.

it seems possible that there is a form of *T. peduncularis* sufficiently distinct as regards morphological characters and chromosome number as to be considered a named variety of the species.

Consideration of further findings in the *Triteleia laxa* complex will be taken up in detail later. Figure 1 shows the 42 meiotic chromosomes of *B. laxa* Blue King which were not clearly distinguished during earlier study. *Triteleia crocea* bloomed in Missouri, and the species determination formerly based on synonomy only was found to be correct. The material labeled *T. hendersoni* bloomed in New Jersey but produced purple flowers resembling those of *B. purdyi*. It is possible that corms of *T. hendersoni* have not been used in these studies and that the data reported for *T. hendersoni* properly belong to some other species.

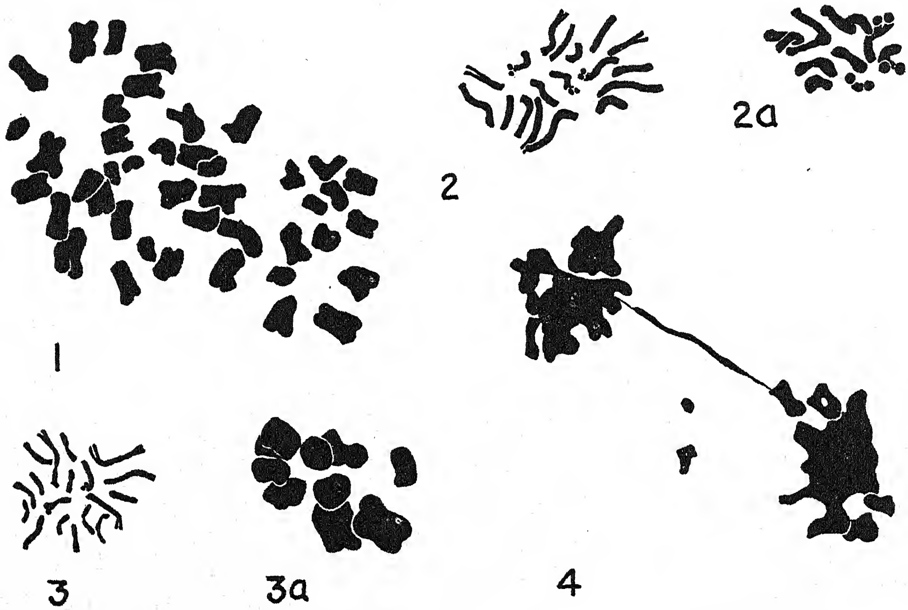
TRITELEIA LAXA COMPLEX

Earlier findings (1) showed that plants called by PURDY *Brodiaea laxa*, *B. laxa* Blue King, and *B. candida* could be separated from one another on the basis of chromosome numbers. Certain morphological differences were noted, but in his discussion of *Triteleia laxa*, HOOVER (5) maintains that there is such great variation of some characters among the forms of *T. laxa* and such constancy in essential characters that it should be regarded as a single taxonomic unit. Thus *B. laxa*, *B. laxa* Blue King, *B. candida*, and other similar forms should all be called *T. laxa*. By keeping individual records of a greater number of plants comprising this "*Triteleia laxa* complex," it was hoped that further conclusions could be reached. HOOVER kindly sent corms of a form of *T. laxa* found in the San Joaquin Valley, California, and additional material of *B. laxa* and *B. laxa* Blue King was purchased from PURDY. Table 2 gives a summary of some of the records kept on these plants grown in the greenhouse in New York City in 1941–42.

The material received from HOOVER as *Triteleia laxa* bloomed sparingly but fitted his description and resembled the plants of *B. candida*, *B. laxa*, and *B. laxa* Blue King with minor exceptions as noted later. Studies of the mitotic and meiotic chromosomes, however, revealed that n = 9 and 2n = 18 (fig. 2, 2*a*), numbers entirely different from those found for other members of this complex.

The differences in vegetative charac-

ters and flower morphology noted earlier within the *T. laxa* complex continued to exist under more careful analysis, but certain characters were common to all the forms. The leaves of *B. candida* were yellow-green rather than the darker green of the other plants. The leaves of *B. laxa* Blue King were wider than those of *B. laxa* on the average, but this did not hold true in every case. There were only slight differences in size and shape of the corollas of the four groups of plants. The color of the corolla varied from lavender to deep purple, but no one form had a distinct and characteristic color. Some pedicels formed an angle with the corolla tube, some did not. One flowering scape of *B. candida* produced flowers which faced all in one direction and were approximately horizontal. This may be the condition upon which PURDY (private communication) bases the separation of *B. candida* from *B. laxa*. The position of the pistil, whether central or along the lower side of the flower, was variable in all four groups. The differences in size and shape of the anthers appears significant. *B. candida* and *T. laxa* had anthers 2.5–4.0 mm. in length which were blunt and rounded at the apex. *B. laxa* and *B. laxa* Blue King had anthers 4.0–7.0 mm. in length, and they tapered upward to a point. Thus in size and shape there is a consistent difference in the morphology of the anthers. In the species description of *T. laxa* (5), HOOVER states that the anthers are 2–5 mm. long. The time of flowering was earlier for *B. candida* and *T. laxa* than for *B. laxa* and Blue King (table 2). Only two corms of Blue King bloomed in February and one in March, the height of bloom being in April. *T. laxa* bloomed late in February, *B. candida* early in March, and *B. laxa* in March and April. The characters common to all forms of



FIGS. 1–4.—Fig. 1, *B. laxa* Blue King, anaphase I. Fig. 2, *T. laxa*, root-tip metaphase. Fig. 2*a*, same, metaphase II. Fig. 3, *Bl. crocea*, root-tip metaphase. Fig. 3*a*, same, metaphase I. Fig. 4, *B. laxa* Blue King, telophase I.

T. laxa, and which determine the species, include the attachment of stamens at two different levels with filaments of about equal length, and perianth blue with the tube attenuate at base and ranging in length from 22 to 38 mm.

Since a variation of the chromosome number for *B. laxa* was noted earlier, an attempt was made to study both somatic and meiotic chromosomes of each plant under observation and note any correlation between chromosome morphology and vegetative and flower characters. The data on this are not complete, but no striking correlations were noted. Of six plants of *B. laxa* for which the somatic chromosome number was determined, three had 30 chromosomes, two had 28, and one appeared to have a variable number, always slightly above 30. Meiotic chromosome counts of three other plants of this form revealed 14 chromosomes in two cases and 14 chromosomes plus extra fragments in the third. The extra fragments, usually but not always visible and one, two, or three in number, were especially apparent at metaphase I, when they lay in the cytoplasm at some distance from the metaphase plate. Their ultimate fate could not be determined. In only one instance was a bit of chromatin seen isolated from the four chromatin masses characteristic of telophase II. Bridging and lagging at both telophase I and II were seen occasionally in the anther smear material of two of the three plants and in two additional plants for which the meiotic count was not determined.

Similar meiotic irregularities were noted in the Blue King material. Bridging as shown in figure 4 was especially common in the plant from which the pictured material was taken. A temporary slide made from an anther of the same plant (fig. 4) contained 31 normal telophase stages and 44 telophase stages in which there were fragments or bridging or both. No irregularities were observed in the material of *T. laxa* and *B. candida* examined.

TABLE 2

DATA ON TRITELEIA LAXA COMPLEX

Species	No. of corms	No. of plants with flowers	Appearance of first flower (1943)	Average		Range		Chromosome number (2n)	No. of plants with meiotic irregularities
				Leaf width (mm.)	Anther length (mm.)	Anther length (mm.)	Corolla length (cm.)		
B. laxa	31	15	3/11–4/25	7.9	5.3	4.0–6.5	2.5–3.8	28–30	5
B. laxa Blue King	33	19	2/9 –5/3	11.5	5.9	4.5–7.0	2.6–3.8	42	6
T. laxa	21	2	2/18–2/28	....	3.0	3.0	2.4–3.2	18	0
B. candida	13	4	3/1 –3/11	....	3.5	2.5–4.0	2.2–3.7	48	0

In the Chicago greenhouses, neither *B. laxa* nor *B. laxa* Blue King set seed. In New York, seven selfings of *B. laxa* resulted in capsules of seed. (Under the term "selfing" is included both the use of pollen from the same flower and of pollen from another flower produced on the same scape.) One selfing and three attempts at crossing two different plants of *B. laxa* yielded no seed. Three selfings of Blue King, two of the same plant, produced seeds, but eight selfings yielded no seed. Six attempts at crossing two different plants of Blue King yielded no seed. Using both *B. laxa* and Blue King as

pollen parents, thirteen crossings were attempted between the plants with negative results. Three attempts to cross *B. candida* and *B. laxa* produced no seed. The cytological data on these plants are not complete enough to determine whether the varying chromosome number of *B. laxa* and the meiotic irregularities of both *B. laxa* and Blue King influence the ability to produce seed. Apparently both forms can reproduce by means of seeds, and crossing between the two is rare or impossible.

Dr. WODEHOUSE kindly examined some of the pollen of the *Brodiaea* plants grown in New York City. He found that the pollen grains of *B. laxa* and *B. laxa* Blue King were almost alike. They differed only in the slightly larger size of the Blue King grains, 70.7 μ as compared with 51.6 μ for *B. laxa*, and the pattern of the exine, which was finely reticulate in the former and merely pitted in the latter.

The findings outlined in the foregoing paragraphs confirm HOOVER's statement that *T. laxa* is a variable species. Whether it is a single species, or whether it contains forms that should be given varietal or species rank, cannot be determined without further field work and cytological analysis of material collected. Such a question cannot be settled by laboratory and greenhouse work and relatively small numbers of plants. *B. laxa* Blue King appears to be a slightly more vigorous form of *B. laxa*. The only "good" characters found in this study upon which separation could be based are the size and shape of anthers, the time of flowering, and the chromosome number. It is unusual to have such 2n chromosome numbers as 18, 28-30, 42, and 48 occur within a single species. Six would seem to be the basic number of such a series. These numbers and the meiotic irregularities observed suggest that the "species" *T. laxa* is a complex still in the evolutionary process of becoming a species.

DICHELOSTEMMA VOLUBILE × D. IDA-MAIA

A capsule of seed was reported (1) following the pollination of a flower of *Dichelostemma volubile* with pollen from *D. ida-maia*. Planted outdoors the following fall, these seeds did not germinate. Forty-one further attempts at cross-pollination between these two species failed to yield a single seed. It is concluded that a cross is not possible and that the earlier seeds were not actually the product of such a cross or were not viable. A misstatement concerning *D. venustum* in this connection was made earlier. *D. venustum* is not a hybrid between *D. volubile* and *D. ida-maia* but has been claimed to be a hybrid between *D. congestum* and *D. ida-maia* (4).

BLOOMERIA CROCEA

HOOVER (5) has stressed the similarities between *Triteleia* and *Bloomeria*. A study of the chromosomes of *B. crocea* does not bear out this close association. The mitotic chromosome number was 18 and the meiotic number 9 (fig. 3, 3*a*). Individual chromosome morphology was not distinct enough to be significant, but the small diameters of the mitotic chromosomes and the number 18 suggest a closer relationship with *Dichelostemma* than with either *Triteleia* or *Brodiaea*. WODEHOUSE found the pollen grains of *Bloomeria crocea* to be quite different from those of *Triteleia*, *Brodiaea laxa*, and *Dichelostemma volubilis*, both in shape and in the texture of the exine.

Summary

1. The species determinations of *Dichelostemma pulchellum* and *Triteleia*

crocea were confirmed by examination of fresh flowers. The identity of the material considered as *Triteleia hendersoni* is doubtful.

2. The existence of a variety of *Triteleia peduncularis*, based on pedicel length, flower color, chromosome number, and perhaps distribution, is suggested.

3. *Triteleia laxa* is considered a species complex made up of many forms. Somatic chromosome numbers include 18, 28, 30, 42, and 48. Reconsideration of the species delimitation seems indicated.

4. *Dichelostemma volubile* and *D. idamaia* are not readily crossed.

5. *Bloomeria crocea* has 18 mitotic and 9 meiotic chromosomes.

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LITERATURE CITED

1. BURBANCK, MADELINE P., Cytological and taxonomic studies in the genus *Brodiaea*. BOT. GAZ. 103: 247–265. 1941.
2. FLORY, W. S., and YARNELL, S. H., A review of chromosome numbers in the Hemerocallideae, Alstroemeriales, and Amaryllidales. Herbertia 4: 163–181. 1937.
3. HOOVER, R. F., A definition of the genus *Brodiaea*. Bull. Torr. Bot. Club 66: 161–166. 1939.
4. ———, The genus *Dichelostemma*. Amer. Mid. Nat. 24: 463–476. 1940.
5. ———, A systematic study of *Triteleia*. Amer. Mid. Nat. 25: 73–100. 1941.
6. HUTCHINSON, J., The families of flower plants. II. Monocotyledons. London. 1934.
7. TISCHLER, G., Pflanzliche Chromosomen-Zahlen. Tab. Period. 12: 57–115. 1937.

NATURE AND RATE OF DEVELOPMENT OF ROOT SYSTEM OF CENTAUREA PICRIS[1]

JOHN C. FRAZIER

Introduction

This is the third in a series of reports on the growth habits of noxious perennial weeds of central United States, the work being conducted by the Kansas Agricultural Experiment Station. A study (2) of the nature and rate of development of field bindweed established the scope and methods of procedure. The second investigation (3) dealt with hoary cress. The present study has to do with Russian knapweed, *Centaurea picris* Pall. (*C. repens* L.), a noxious weed of potential significance rivaling that of field bindweed and hoary cress in this area.

Environmental conditions and methods

SOIL DATA.—All plants[2] considered in this paper were taken from soil, the profile of which was described by FRAZIER (3). No layer of this profile appears to be of such nature as to impede seriously the development of the roots of deep-rooting plants.

METEOROLOGICAL DATA.—The monthly and annual temperature and precipitation data for the first 10 months of 1942 compared with the long-time average of these factors are given in table 1. The summer of 1942 had temperatures of 100° F. or higher for 5 days, as compared with 38 such days in 1937—the year the comparable study was made on field bindweed, and 15 such days in 1941—the year hoary cress was investigated. The average annual number of days having

[1] Contribution no. 454, Department of Botany, Kansas Agricultural Experiment Station.

[2] The term plant as used in this paper refers to all the growth from a single seed. The term shoot is applied to the individual leafy stems commonly called plants in control studies.

this temperature during the 50 years, 1889–1938, is 15. The average monthly precipitation for the first 10 months of 1942 was 3.81 inches, as compared with the monthly average of 2.86 for these months during the 83-year period, 1858–1940. Much of the precipitation for the first 10 months of 1942 fell as torrential rains. There was a 24-hour period in May with 1.75 inches; two in June with 1.77 and 2.85 inches, respectively; and one in September with 3.25 inches.

TABLE 1

MONTHLY AND ANNUAL TEMPERATURES (IN °F.) AND PRECIPITATION (IN INCHES) AT MANHATTAN, KANSAS, 1942

Month	Temperature		Precipitation	
	Mean*	85-year average (1856–1940)†	Total*	83-year average (1858–1940)†
Jan.	30.2	27.0	0.64	0.75
Feb.	32.7	31.5	0.93	1.14
Mar.	44.9	42.5	1.61	1.48
Apr.	60.4	54.5	1.77	2.67
May	63.4	64.6	6.00	4.31
June	73.2	74.5	11.48	4.50
July	80.8	79.5	2.19	4.25
Aug.	77.7	77.5	3.85	3.89
Sept.	67.1	69.3	7.45	3.44
Oct.	59.0	56.5	2.20	2.16
Annual mean or total	55.6	54.2	41.14	30.97

* Meteorological data from U.S. Weather Bureau, Kansas section.

† Computed by Dr. A. B. CARDWELL of Kansas State College.

METHODS.—The plants were grown on a small plot of land free of both noxious weeds and sodium chlorate. The soil is a fairly typical Geary silt loam, representative of the wind-deposited soil of the upland of this locality. The plot had been cultivated a decade earlier but had not been disturbed recently.

The first planting was made April 11, 1942. Several seedlings emerged on April 22. A plant removed April 29 was designated no. 1. It illustrates a seedling 1 week after emergence. A second planting was made April 18. In each planting, twenty seeds were placed $\frac{3}{8}$ inch below the surface at each of twenty-one points so spaced that seven of the plantings were 13 feet apart and the remainder were separated by 5–8 feet. Emergence of the second planting began April 27. On April 30, seedlings emerged at sixteen of the twenty-one points. These sixteen seedlings were retained but all others were removed, including those emerging after that date. All plants used except no. 1 emerged on April 30. The area was kept free of all other vegetation, so that the only competition for water and nutrients, if any existed, was among the knapweed plants.

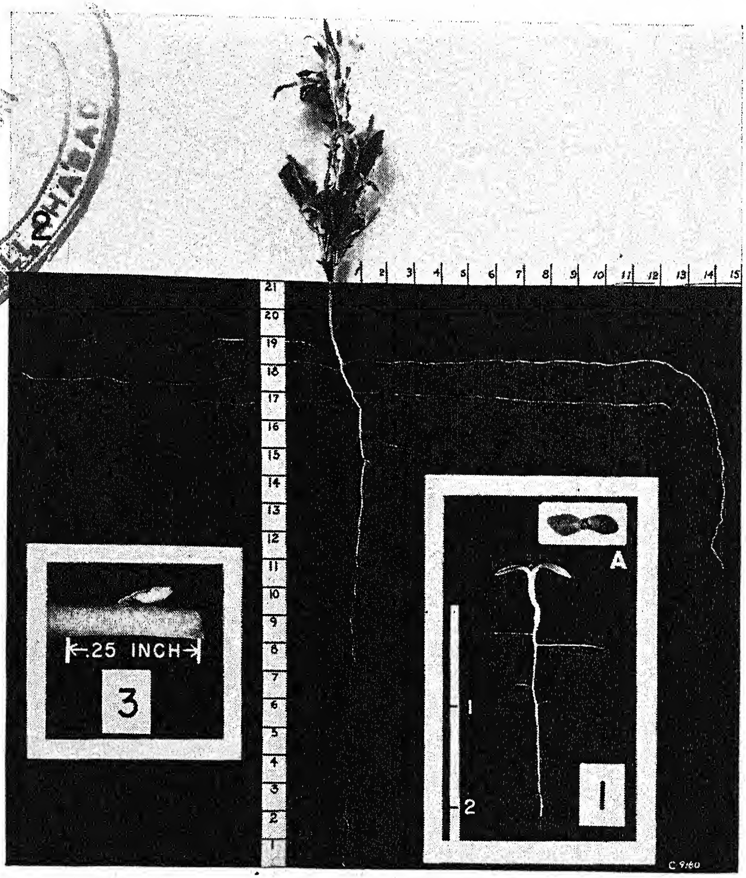
The root systems were excavated by a modification of the trenching method developed by WEAVER (7) as described by FRAZIER (2). Efforts were made to keep the root system as one organic entity; if a root broke, the ends were immediately tied together. Measurements of the plant parts were made and recorded, so that as illustrated they occupied the same relationship, one part to another, as they did in the soil, except that in figures 1, 2, 4, and 5 all lateral roots are arranged in one plane. While as many as possible of the finer roots were obtained, it is not contended that a great portion of them was secured.

Observations

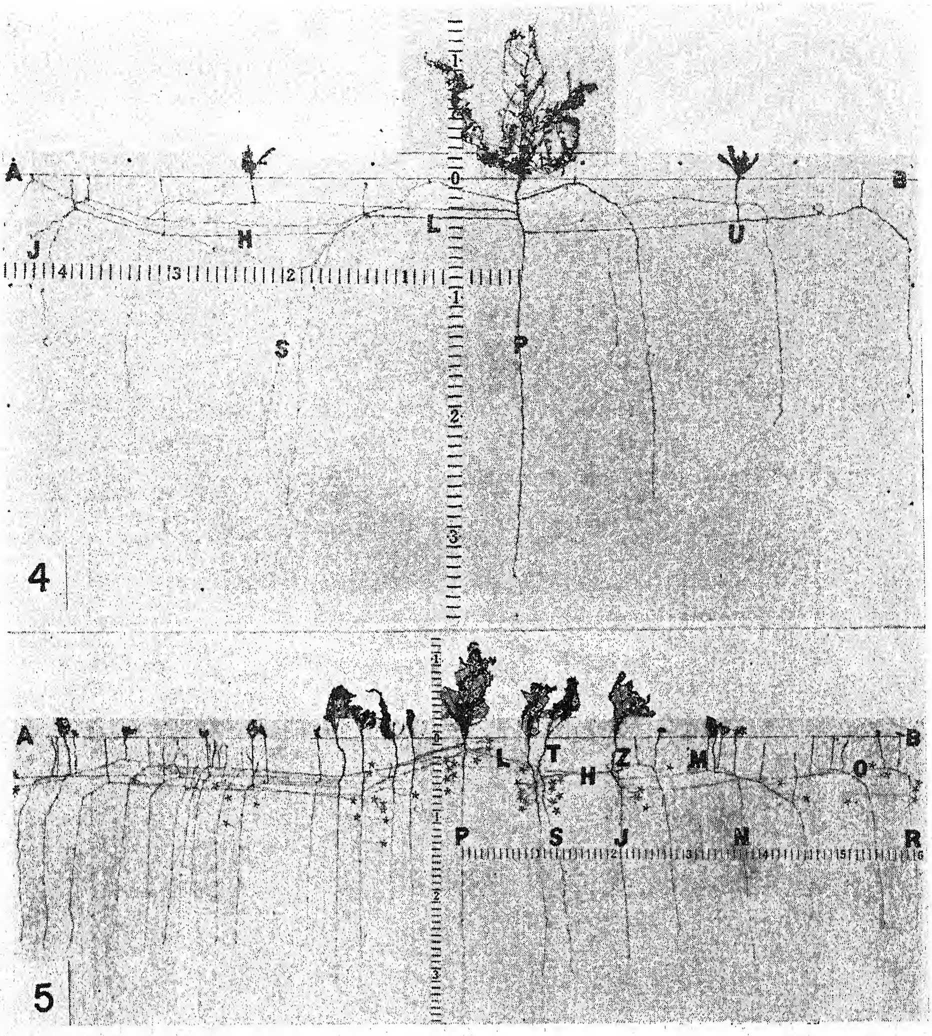
Eight plants were excavated weekly between April 29 and July 16, except for the second, fifth, and tenth weeks after emergence, and plants 9, 10, and 11 excavated August 6, September 17, and in early November, 1942, respectively. A twelfth plant was excavated in mid-September, 1943. Figures 1, 2, 4, and 5 show the vertical penetration and the

radial spread of roots of representative plants. Data of the twelve excavations are given in table 2.

By the eighth week after emergence, the general plan of the root system was beginning to be established (fig. 2). Plant no. 10, taken the twentieth week after emergence (fig. 4), showed particularly well the gross morphological nature of the root system. A taproot rapidly penetrated directly downward from the germinating seed. It was a primary root in order of development and vertical in position; hence it is designated the primary vertical root (fig. 4*P*). Many branch roots arose throughout the length of this taproot, most of them small feeding roots. A few, probably those more favorably situated in relation to soil moisture and nutrients, grew extensively and became permanent parts of the root system. They are designated permanent lateral roots of the first order (fig. 4*L*). These grew horizontally 12–36 inches and then turned down and were from that point vertical taproots, desig-



FIGS. 1–3.—Stages in development of plants of Russian knapweed. Fig. 1, seedling 1 week after emergence; *A*, view of cotyledons from above. Fig. 2, young plant 8 weeks after emergence showing permanent lateral turning down to become secondary vertical root. Fig. 3, root-borne stem bud, $\times 3$, on primary permanent root of plant 11 weeks after emergence. Scales in inches.



FIGS. 4, 5.—Fig. 4, complete plant 20 weeks after emergence (*A–B* is ground line). *P*, primary vertical; *L*, permanent lateral of first order; *S*, secondary vertical of first order; *H*, permanent lateral of second order; *J*, secondary vertical of second order; *U*, rhizome arising from lateral root. Fig. 5, approximately 60% of growth attained by one plant 7 months after emergence, arranged to show place relationship of parts essentially as in the soil. Stars indicate points from which laterals have been removed for clarity. *M*, permanent lateral of third order; *N*, secondary vertical of third order; *O*, permanent lateral of fourth order; *R*, secondary vertical of fourth order; *T*, *Z*, heavy rhizome and shoot development on or near juncture of permanent lateral and secondary vertical roots. Scales in feet and inches.

nated secondary vertical roots[3] (fig. 4*S*). The permanent lateral roots of any order, which are usually smallest in diameter at their origin, were always in the surface foot, more commonly in the upper 7 inches of the soil. The lateral-vertical roots of the first order gave rise to branch roots from any part of the horizontal or being away from the primary vertical, and turned down to form secondary verticals of the second order (fig. 4*J*). This development was repeated many times during the growing season to give rise to lateral-vertical roots of the third, fourth, and even higher orders (fig. 5). It is by such series of permanent laterals

TABLE 2

RATE AND NATURE OF DEVELOPMENT* AFTER SEEDLING EMERGENCE OF ROOTS OF RUSSIAN KNAPWEED. MANHATTAN, KANSAS, 1942

Plant no.	Weeks after emergence	Maximum vertical penetration (inches)	Maximum radial spread (inches)	Plant condition
1	1	$2\frac{1}{4}$	$\frac{5}{8}$	Typically shaped cotyledons (fig. 1, 1A)
2	3	$9\frac{1}{2}$	$3\frac{1}{8}$	First true leaves
3	4	14	$5\frac{3}{4}$	Additional true leaves beginning to form rosette; cotyledons lost
4	6	$17\frac{1}{8}$	$7\frac{1}{2}$	Additional true leaves
5	7	$19\frac{1}{8}$	9	Rosette of leaves well developed
6	8	21	$14\frac{1}{2}$	Lateral roots beginning to grow downward at tip to become secondary verticals (fig. 2)
7	9	$17\frac{1}{2}$	12	Several secondary vertical roots grown downward approximately 12 inches
8	11	$31\frac{1}{2}$	22	First root-borne stem buds on lateral roots 16–17 inches from primary vertical root (if underground, these develop into rhizomes)
9	14	$31\frac{1}{4}$	22	Rhizomes on lateral roots giving rise to emerging leafy shoots
10	20	44	$59\frac{1}{2}$	Many rhizomes reaching soil surface and giving rise to well-developed shoots (fig. 4)
11	28	$45\frac{1}{2}$	78	Root development including permanent lateral and secondary verticals through fifth order (fig. 5)
12	72 (1943)	$10\frac{1}{2}$ ft.	11–12 ft. (at least)	Secondary vertical roots apparently penetrating deeper than primary verticals and larger in diameter, particularly in first 12–24 inches below bend where lateral root becomes secondary vertical

* First flowering occurred July 7, 1943.

vertical growth. Most of these are small feeding roots. However, at or just below the bend where the permanent lateral of the first order becomes a secondary vertical root, permanent laterals of the second order developed (fig. 4*H*). These extended horizontally 6–22 inches from the point of origin, the general direction that horizontal spreading of the plant is accomplished. If more than one permanent lateral arose from the place of origin of such roots of the second or higher orders, they radiated from the point of origin; but, in general, all maintained growth away from the primary vertical. The result was that the plant occupied a much higher proportion of the ever-increasing area, somewhat circular in shape, than it would have otherwise. Concurrent studies indicated that plants

[3] The horizontal and vertical phases of these roots are included in the term lateral-vertical. The vertical phase is considered to begin where the slender lateral enlarges markedly.

growing under conditions of severe competition made less lateral development in unit time.

All shoot development, except that from the plumule, was derived from root-borne stem buds. These developed on any part of the permanent root system (that is, the permanent laterals and the two types of vertical roots). Eleven weeks after first seedling emergence, a number of these buds had formed on or near the bend between the permanent lateral and the secondary vertical roots. A few also developed on the permanent laterals. One of the latter is shown in figure 3. By 14 weeks after seedling emergence such buds had produced rhizomes, some of which had grown to a length of 2 inches. Two of them initiated shoots upon reaching the surface. By 20 weeks after seedling emergence, shoots from rhizomes were well developed (fig. 4). The location of the most extensive shoot development from root-borne stem buds was at or near the bend between the lateral root of any order and its vertical phase (fig. 5*T*, *Z*). Infrequently, shoots not subtended by a vertical root were found along the permanent laterals (fig. 4*U*). Regardless of where these buds formed, they gave rise to rhizomes unless they were borne at the ground line, in which case they gave rise to leafy shoots.

Twenty-eight weeks after it had emerged as a seedling, a plant (fig. 5) had sent its primary vertical root to a depth of $45\frac{1}{2}$ inches and had produced sixteen permanent lateral roots of the first order. The latter were arranged radially around the primary vertical. Fourteen of these laterals had developed secondary vertical roots. Eleven of the fourteen had given rise to lateral-secondary verticals of the second and higher orders. Seven of the eleven are shown in figure 5. There were twenty-eight regions of shoot development on the fourteen lateral roots which had developed secondary verticals. The shoot development of these twenty-eight regions came from seventy-one rhizomes, each of which had developed from a root-borne stem bud. There were no rhizomes or root-borne buds on the two laterals which had not formed secondary vertical roots. The growth attained 28 weeks after emergence was so carefully removed that practically all the plant was recovered. Approximately 60% of the growth constituting the plant was arranged to show the place relationship of these parts essentially as they were in the soil (fig. 5).

Discussion

The rate of growth of plants of Russian knapweed from seed under known soil and climatic conditions, with little or no competition, is not to be construed as representing their growth rate under all conditions. It illustrates only the growth potential in a favorable noncompetitive situation.

The observations of Ball and Robbins (1) on the general nature of the root system of this plant agree in general with the present findings. However, Ball and Robbins consider the horizontal axes of the root system as "creeping, perennial rootstocks," on which "close observation . . . . shows small, narrow, appressed scale leaves, arranged alternately at regular intervals." In the study here reported, numerous observations, both macroscopic and microscopic, show the horizontal axes to be roots and not underground stems, as the term rootstock used by Ball and Robbins implies. Rogers (5) considered the horizontal axes to be roots. Buds (fig. 3) do arise adventitiously at irregular intervals on the horizontal roots, and less commonly on the upper portions of their vertical extensions (secondary vertical roots), but they are not subtended by scale

leaves. The horizontal axes are roots, not rootstocks (underground stems), in field bindweed and hoary cress—weeds with a root system identical in general form with that of Russian knapweed. KENNEDY and CRAFTS (4) reported this fact for field bindweed, SIMONDS (6) for hoary cress, and FRAZIER (2, 3) confirmed the finding.

Research to determine why certain of the branch roots develop more extensively than others and become permanent lateral roots has not been undertaken. It has been conjectured by KENNEDY and CRAFTS (4) for field bindweed and by FRAZIER (3) for hoary cress that these variations result from differences in the supplies of soil moisture and plant nutrients.

Summary

1. Plants of Russian knapweed, grown from seed on a typical upland loam soil at Manhattan, Kansas, under known temperature and precipitation conditions and not subject to competition, were studied from the seedling stage through 72 weeks of growth.

2. The root system of well-established plants consisted of the original root (primary vertical), one to many permanent lateral roots, and their vertical extensions (secondary vertical roots).

3. The plants spread horizontally by series of permanent lateral roots. The permanent laterals of the first order arise on the primary vertical root. Unless injury or too severe competition prevents, successive orders of permanent laterals arise at or near the bend where a permanent lateral of the preceding order turns down to become a vertical (secondary vertical root).

4. The plants had spread radially $6\frac{1}{2}$ feet in one growing season, and 11–12 feet by the end of the second season, at which time several vertical roots had reached a depth of $10\frac{1}{2}$ feet.

5. The source of shoot development, other than that arising from the plumule, was from root-borne buds which produce either leafy shoots directly (if at the ground line), or rhizomes (if below ground) which in turn give rise to leafy shoots. These buds arose in greatest abundance at or near the bend separating the permanent lateral of any order from its vertical phase. The shoot development of old plants is wholly from root-borne buds.

6. The general type of development is the same as that of field bindweed and hoary cress. The rate of development of knapweed appeared to be about the same as that of hoary cress for the first 72–75 weeks. After the first 8–10 weeks following emergence, both plants developed at a somewhat slower rate than bindweed. Bindweed flowered during the first season of growth, while hoary cress and knapweed did not flower until the second season.

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LITERATURE CITED

1. BALL, W. S., and ROBBINS, W. W., Russian knapweed. California Dept. Agr. Monthly Bull. 20:666–668. 1931.
2. FRAZIER, J. C., Nature and rate of development of root system of *Convolvulus arvensis*. BOT. GAZ. 104:417–425. 1943.
3. ———, Nature and rate of development of root system of *Lepidium draba*. BOT. GAZ. 105:244–250. 1943.
4. KENNEDY, P. B., and CRAFTS, A. S., The anatomy of *Convolvulus arvensis*, wild morning-glory or field bindweed. Hilgardia 5:591–622. 1931.
5. ROGERS, C. F., Canada thistle and Russian knapweed and their control. Colorado Agr. Exp. Sta. Bull. 348. 1928.
6. SIMONDS, A. O., The anatomical development of *Lepidium draba*. Jour. Agr. Res. 57:917–928. 1938.
7. WEAVER, J. E., Root development of field crops. McGraw-Hill, New York. 1926.

EFFECT OF VARYING NUTRITIONAL TREATMENTS ON GROWTH AND RUBBER ACCUMULATION IN GUAYULE

JAMES BONNER

Introduction

Preliminary experiments conducted in 1941 suggested that rubber accumulation in guayule (*Parthenium argentatum* Gray) plants might be influenced significantly by the nature and amount of available nutrients. Two experiments designed to explore this possibility were conducted during 1942–43. In the first, plants were supplied with thirty-eight different nutrient solutions of varying anion or cation composition planned according to a triangular system (2). In the second experiment, young plants were supplied with nitrogen, either as nitrate, as ammonium, or as mixtures of the two forms. Each experiment lasted through an entire growing season of 8.5 months, and since they were conducted outdoors, the plants were exposed to the changing environments of summer, fall, and winter. In each case two harvests were made, one at the end of the summer, when rubber content of the plants was low, and one the following spring, when rubber content had reached its maximum for the season.

Material and methods

The planting stock consisted of nursery seedlings[1] grown by the American Rubber Producers, Inc., and supplied by the U.S. Forest Service. The plants had the tops removed close to the crown and were without leaves. In addition, they were small and weighed an average (dry) of 60 mg. per plant. During the first week in June this material was planted in 4-mesh gravel contained in 1.5-gallon cans provided with drainage and which had been previously painted with a nontoxic asphaltum paint. Three plants were placed in each of approximately 1000 cans. After 7–10 days, when new shoot growth had begun to appear, one plant was removed from each can, leaving the two most uniform. On June 15, 1942, 604 containers of plants selected for uniformity were chosen for initiation of the first experiment.

Experiment I

Nutrient solutions.—This experiment was divided into two parts, one in which nutrient solutions of varying anion composition were applied and a second in which solutions of varying cation composition were applied. Six stock solutions, whose compositions are given in table 1, were prepared (2). Three of these stock solutions each contained one major anion but had similar composition as to cations. The other three each contained one major cation but had similar composition as to anions. The three anion solutions were combined by ninths to give nineteen of the fifty-five possible combinations, while the three cation solutions were similarly combined to give nineteen further combinations. Figure 1*A* shows the positions in the triangles occupied by the thirty-eight solutions. Table 2 shows the proportions of the six stock solutions used in making up the thirty-eight nutrient solutions. The anion solutions maintain constant ratios between cations (table 1).

The stock solutions were made with chemicals of technical or U.S.P. grade.

[1] Owing to critical shortage of guayule plants in the spring of 1942, the U.S. Forest Service was able to supply only cull nursery stock.

All solutions were made with tap water, and the plants were watered with tap water. Nutrient was applied at the rate of 500 cc. per culture three times per week, and the plants were further watered as required.

EXPERIMENTAL DESIGN.—The anion and cation treatments, each including nineteen nutrients, were tested in each of four plots. In each plot the nineteen treatments were randomized, and each treatment was tested with four replicate cultures. Thus, any one treatment was tested in four plots, each containing four cultures (each of two plants), or a total of thirty-two plants per treatment. The data were treated throughout by the analysis of variance, and summaries of the treatments appear in the tables. The cation and anion portions of the experiment were set up in separate but adjoining locations and are not strictly comparable, as is evident from the data.

HARVEST OF SEPTEMBER 15.—On September 15, after 3 months of treatment, half the cultures were harvested. Each treatment was represented in this harvest by sixteen plants; that is, by two plants from each of two cultures from each of four plots. The plants were washed free of gravel, dried rapidly at 65° C., and weighed (the leaves separately from the woody portion of stems and roots). This woody portion was then ground in a Wiley mill and analyzed for rubber and resin by the solvent-extraction method.

TABLE 1

CONCENTRATIONS OF STOCK SOLUTIONS I–VI IN MILLIEQUIVALENTS OF INDIVIDUAL IONS PER LITER*

Salt	I NO_3	II SO_4	III H_2PO_4	IV Mg	V K	VI Ca
KNO_3	4.5				4.5	
$Ca(NO_3)_2$	12.0					12.0
$Mg(NO_3)_2$	9.0			9.0		
K_2SO_4		4.5			4.5	
$CaSO_4$		12.0				12.0
$MgSO_4$		9.0		9.0		
KH_2PO_4			4.5		4.5	
$Ca(H_2PO_4)_2$			12.0			12.0
$MgHPO_4$			9.0	9.0		

* Each stock solution contained in addition: 0.5 p.p.m. B as H_3BO_3, 0.5 p.p.m. Mn as $MnSO_4$, 0.1 p.p.m. Zn as $ZnSO_4$, 0.02 p.p.m. Cu as $CuSO_4$, 0.01 p.p.m. Mo as H_2MoO_4, and 1.0 p.p.m. Fe as ferrous tartrate.

TABLE 2

RATIOS OF STOCK SOLUTIONS I–VI USED IN MAKING UP NUTRIENT SOLUTIONS FOR EXPERIMENT I. FIGURES ARE PARTS OF INDIVIDUAL STOCK SOLUTIONS PER NINE PARTS OF NUTRIENT

Nutrient solution	I NO_3	II SO_4	III H_2PO_4	Nutrient solution	IV Mg	V K	VI Ca
1	9	0	0	21	9	0	0
2	7	1	1	22	7	1	1
3	6	3	0	23	6	3	0
4	6	0	3	24	6	0	3
5	5	2	2	25	5	2	2
6	4	4	1	26	4	4	1
7	4	1	4	27	4	1	4
8	3	6	0	28	3	6	0
9	3	3	3	29	3	3	3
10	3	0	6	30	3	0	6
11	2	5	2	31	2	5	2
12	2	2	5	32	2	2	5
13	1	7	1	33	1	7	1
14	1	4	4	34	1	4	4
15	1	1	7	35	1	1	7
16	0	9	0	36	0	9	0
17	0	6	3	37	0	6	3
18	0	3	6	38	0	3	6
19	0	0	9	39	0	0	9

Figure 2*A* gives data on the dry weight of stems and roots for the nineteen anion treatments, while figure 2*B* gives data concerning the leaf dry weights for the same treatments. Results of analysis-of-variance treatments for the two sets of data are given in table 3. Significant differences in dry weights of both the woody portion and

the leaves produced in the nutrient treatments are apparent. Of the deficient plants, those which received low nitrate were the smallest. These were stunted, possessed few and yellow leaves, and flowered meagerly. A less well-marked response to phosphate deficiency was evident. Phosphate-deficient plants showed a slight tendency to anthocyanin formation. No marked response to sulphate deficiency took place under the conditions used. Figure 3*A* and 3*B* give data on the resin and rubber contents, respectively, of the plants produced in the nineteen anion treatments, while table 3 summarizes the results of the variance analysis. Resin concentration varied but 25% among the several treatments, and the analysis shows that this variation is not significant. The rubber concentrations were all low, as is also true of fieldgrown guayule harvested early in the fall. Still lower rubber concentrations would have been obtained had the plants been harvested 2–3 weeks earlier (about the first of September). Other experiments conducted during the same time and under the same conditions showed a rapid increase in rubber concentration during the first 2 weeks of September. Rubber concentration, though low, was significantly affected by anion nutrition, in particular by the nitrate level. Plants which received either much or very little nitrate contained significantly lower rubber concentrations than plants supplied with nitrate at an intermediate level. No specific influence of phosphate or of sulphate level on rubber concentration was evident.

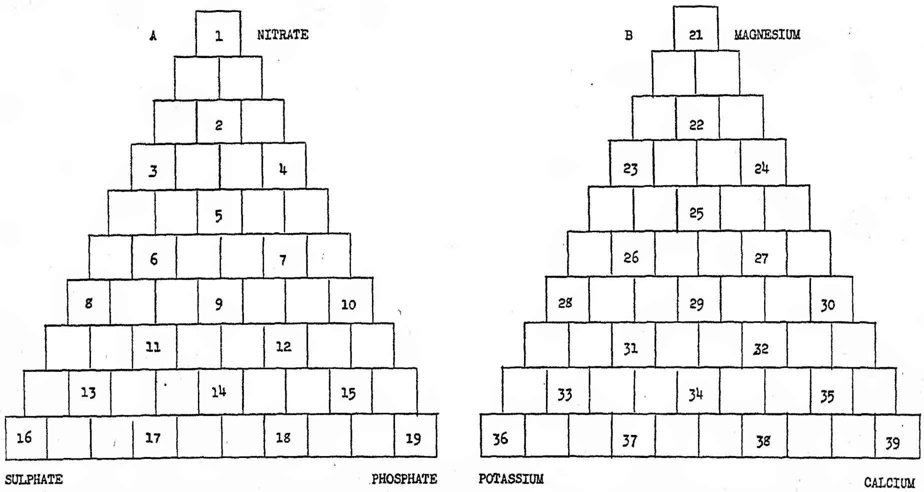
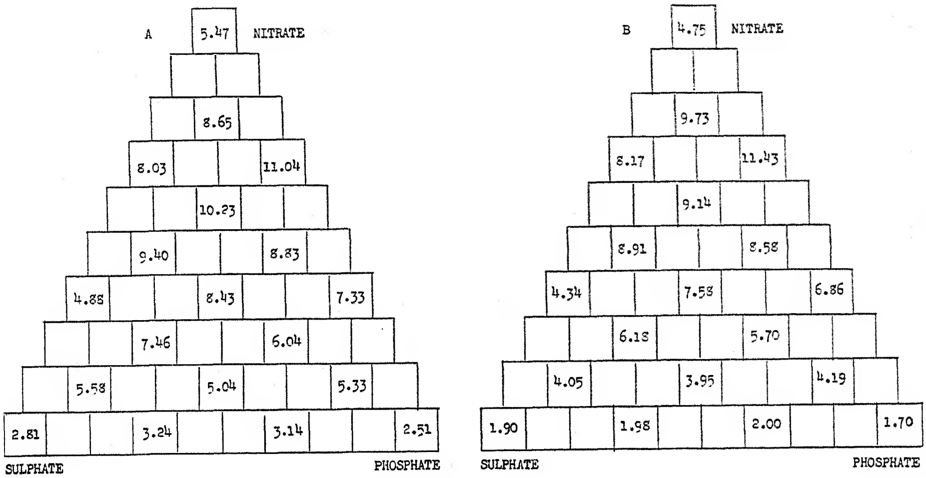


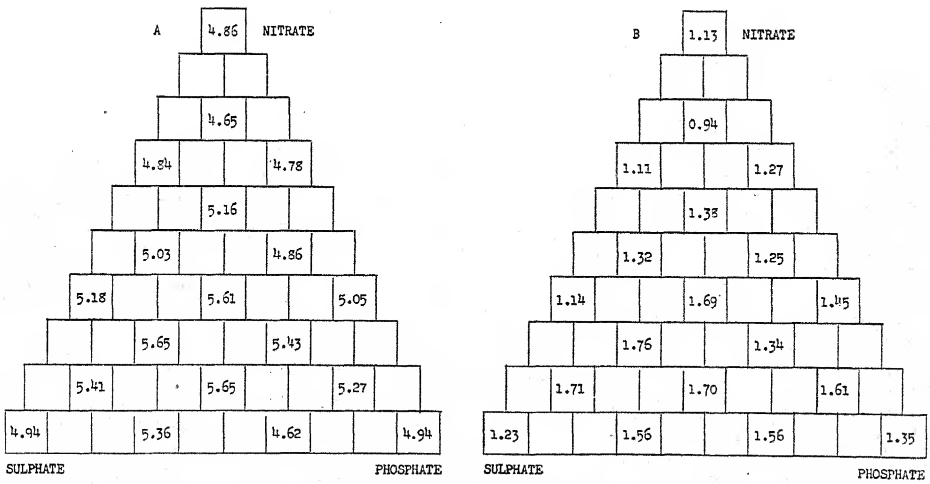
FIG. 1.—*A*, disposition of the 19 nutrient solutions of varying anion composition chosen for test. *B*, same for cation solutions.

In general, treatments tending to produce the largest plants tended also to produce the lowest rubber concentrations. Treatment 2 in particular resulted in more succulent plants, with taller shoots and more impressive bulk, than any other treatment. Treatment 2 also yielded the minimum rubber concentration among the nineteen anion treatments. On the other hand, the smallest plants did not tend to yield the highest concentrations.

Figures 4 and 5 give data on the plants harvested from the nineteen cation

treatments. It is evident that all the responses were smaller than those obtained to varying anion concentrations. Severe deficiencies were not obtained, although there were significant differences in dry weight of stems and roots, dry weight of leaves, and in rubber concentration (table 3). Resin concentration was not significantly affected by the cation treatments. As in the plants of the anion treatments, rubber concentration was low throughout but tended to be somewhat higher in the high than in the low potassium cultures. The lowest rubber concentration occurred in cultures low in both potassium and magnesium but high in calcium.





FIGS. 2, 3.—Harvest of September 15. Fig. 2 (above), dry weight (in grams per two plants produced in the 19 anion treatments) of *A*, stems and roots and *B*, leaves. Difference needed for significance: *A*, 1.34 at 5% level, 1.77 at 1% level; *B*, 1.20 at 5% level, 1.60 at 1% level. Fig. 3 (below), concentration (in percentage dry weight of stems and roots produced in the 19 anion treatments) of *A*, resin; *B*, rubber. Difference needed for significance: *A*, 0.73 at 5% level, 0.97 at 1% level; *B*, 0.40 at 5% level, 0.53 at 1% level.

HARVEST OF MARCH 1.—Experiment I

was continued until March 1, 8.5 months from its initiation and 5.5 months from the first harvest. During the interval between the two harvests, from October through February, the plants of all treatments assumed a dormant aspect. No new shoot growth occurred and no new leaves appeared. The older leaves were shed or knocked off by rain, and total leaf weight decreased. On March 1, new shoot growth began visibly to be initiated, and flower buds were evident on a few plants. Experiments to be reported elsewhere have shown, as noted earlier in this paper, that in the Pasadena area rapid rubber accumulation proceeds through the fall and winter and that the maximum rubber concentration for the season is to be found just at the onset of the spring growing season. Consequently, the rubber concentrations in the plants of experiment I when harvested on March 1 represent the highest values which would be attained in the first season under the conditions used. As with the harvest of September 15, each treatment was represented in the March 1 harvest by sixteen plants; that is, by two plants from each of two cultures from each of the four plots. The plants were dried, weighed, and analyzed as before.

TABLE 3

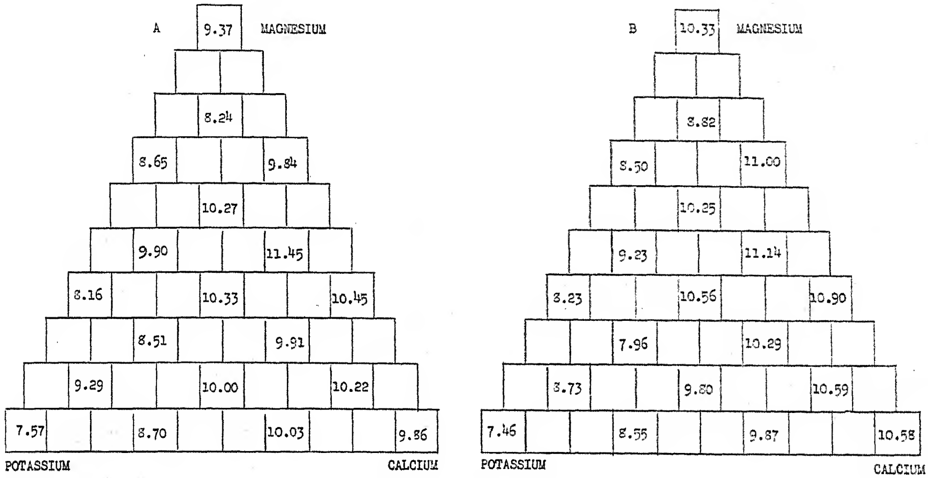
SUMMARY OF F VALUES, DERIVED FROM TREATMENT BY ANALYSIS OF VARIANCE, OF DATA ON GUAYULE PLANTS. EXPERIMENT I

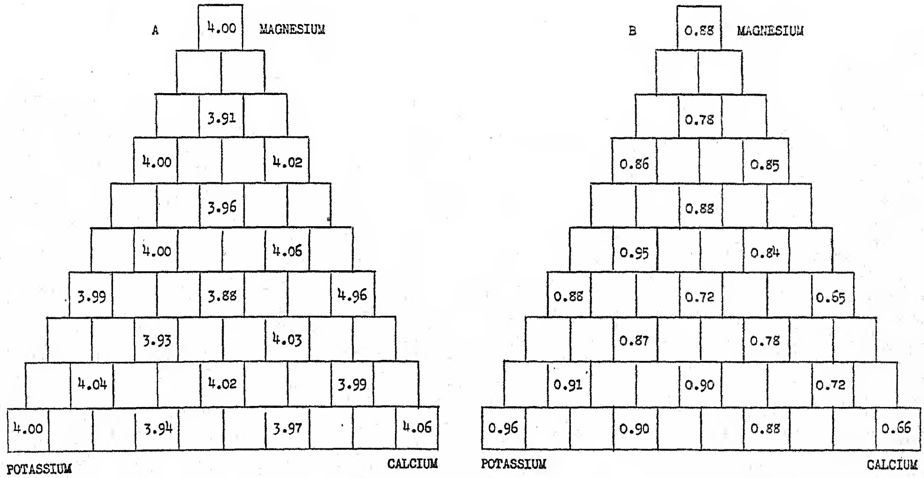
Character	F values: Treatments × error	F values: Blocks × error
	Anion treatments 1–19; September 15 harvest	
Dry wt. of stems and roots	29.90*	6.00*
Dry wt. of leaves	49.10*	3.21†
Resin (%)	1.71	12.71*
Rubber (%)	3.32*	2.43
	Cation treatments 21–39; September 15 harvest	
Dry wt. of stems and roots	2.18†	4.70*
Dry wt. of leaves	3.69*	4.08*
Resin (%)	0.37	19.24*
Rubber (%)	4.02*	7.80*
	Anion treatments 1–19; March 1 harvest	
Dry wt. of stems and roots	102.65*	10.48*
Dry wt. of leaves	53.80*	13.91*
Resin (%)	3.60*	3.00*
Rubber (%)	37.05*	0.89
	Cation treatments 21–39; March 1 harvest	
Dry wt. of stems and roots	3.48*	12.81*
Dry wt. of leaves	2.72*	1.67
Resin (%)	2.89*	0.68
Rubber (%)	3.40*	6.03*

* Significant at 1% level. † Significant at 5% level.

Figure 6 gives data on the stem and root dry weights and the leaf dry weights of the plants grown in the nineteen anion treatments. During the 5.5 months' interval between the two harvests the plants roughly doubled in dry weight of stem and roots but decreased in dry weight of leaves. A winter loss of the older leaves has been observed in other experiments, both in the field and with gravel cultures maintained outdoors. As in the earlier harvest, significant differences in dry weights of stems and roots resulted from the anion treatments (table 3). Nitrogen deficiency produced the smallest plants. Phosphate deficiency appeared only in solution 3 and was not evident at lower nitrogen levels. Sulphate deficiency was not apparent. The largest plants—both as to dry weight of stems

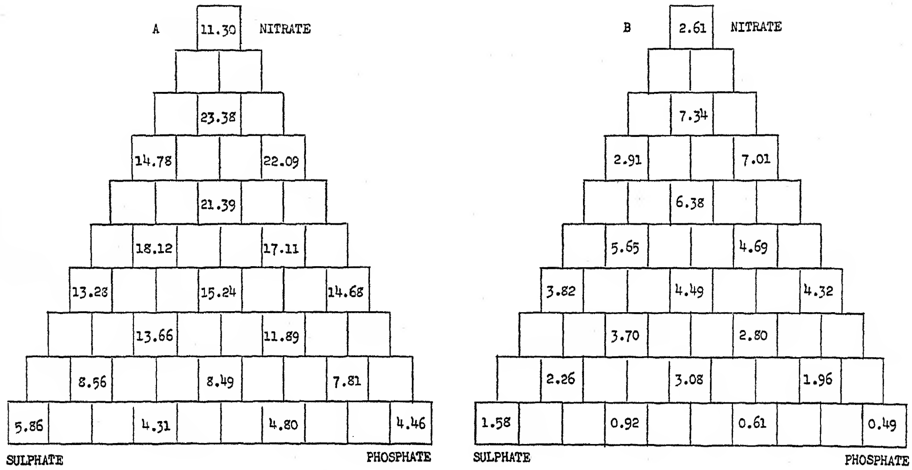
and roots and as to dry weight of leaves—were produced in treatments 2, 4, and 5, which were high in nitrogen and low to moderately high in phosphate but low or very low in sulphate. Figure 7 gives the resin and rubber concentrations found in the plants of the nineteen anion treatments. Significant differences, both in resin and in rubber concentrations, resulted from the varying anion treatments (table 3). As at the September harvest, resin concentration was less influenced than was rubber concentration by the treatments. Thus the largest resin concentration (6.12% in treatment 16) was only 1.2 times as large as the

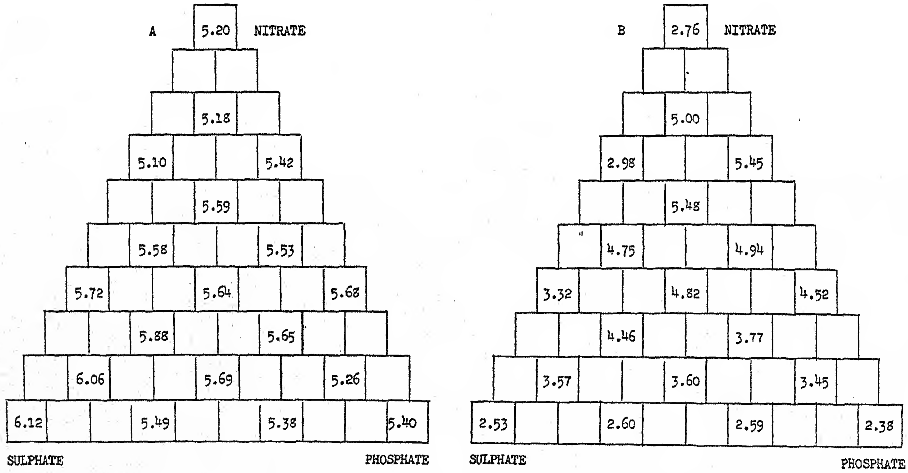




FIGS. 4, 5.—Harvest of September 15. Fig. 4 (above), dry weight (in grams per two plants produced in the 19 cation treatments) of *A*, stems and roots and *B*, leaves. Difference needed for significance: *A*, 1.86 at 5% level, 2.47 at 1% level; *B*, 1.66 at 5% level, 2.21 at 1% level. Fig. 5 (below), concentration (in percentage dry weight of stems and roots produced in the 19 cation treatments) of *A*, resin; *B*, rubber. Difference needed for significance: *A*, 0.36 at 5% level, 0.48 at 1% level; *B*, 0.14 at 5% level, 0.19 at 1% level.

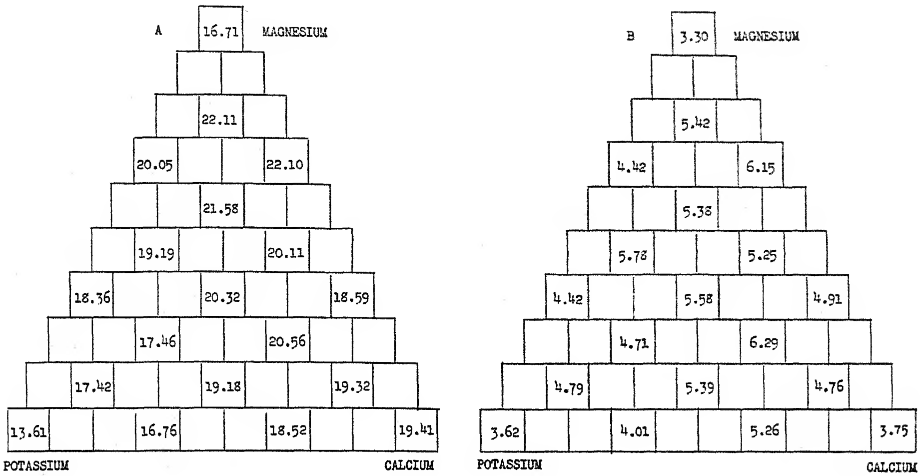
lowest resin concentration (5.10% in treatment 3). The highest rubber concentration (5.48% in treatment 5), however, was 2.3 times as large as the lowest rubber concentration (2.38% in treatment 19). It is also noteworthy that, although rubber concentrations increased markedly during the interval between the two harvests, the resin concentrations increased but little. Comparison of figures 6 and 7 shows that the treatments resulting in the greatest dry weights also resulted in the highest rubber concentrations, while the treatments which resulted in the lowest dry weights resulted in the lowest rubber concentrations. Lower rubber concentration attended both nitrogen and phosphate deficiencies

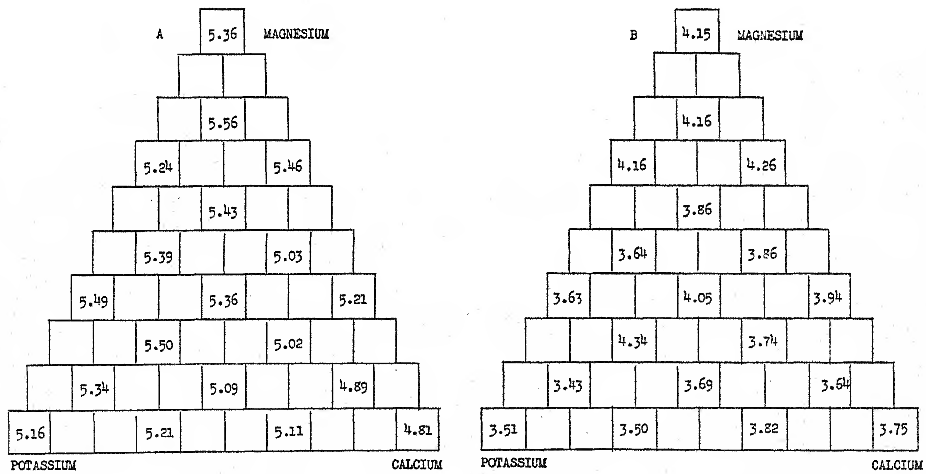




FIGS. 6, 7.—Harvest of March 1. Fig. 6 (above), dry weight (in grams per two plants produced in the 19 anion treatments) of *A*, stems and roots and *B*, leaves. Difference needed for significance: *A*, 1.67 at 5% level, 2.22 at 1% level; *B*, 0.79 at 5% level, 1.05 at 1% level. Fig. 7 (below), concentration (in percentage dry weight of stems and roots produced in the 19 anion treatments of *A*, resin; *B*, rubber). Difference needed for significance: *A*, 0.41 at 5% level, 0.55 at 1% level; *B*, 0.49 at 5% level, 0.65 at 1% level.

(at high level of nitrogen). The correlation of high rubber concentration with great plant growth is not in agreement with the results of the September 15 harvest, where great plant growth tended to be more nearly inversely related to rubber concentration. Comparison of the results of the September with those of the March harvest shows that rubber concentrations early in the season need not be a reliable index to rubber concentrations of similar plants later in the rubber-accumulating season.





FIGS. 8, 9.—Harvest of March 1. Fig. 8 (above), dry weight (in grams per two plants produced in the 19 cation treatments) of *A*, stems and roots and *B*, leaves. Difference needed for significance: *A*, 3.14 at 5% level, 4.12 at 1% level; *B*, 1.42 at 5% level, 1.89 at 1% level. Fig. 9 (below), concentration (in percentage dry weight of stems and roots produced in the 19 cation treatments of *A*, resin; *B*, rubber). Difference needed for significance: *A*, 0.36 at 5% level, 0.47 at 1% level; *B*, 0.41 at 5% level, 0.55 at 1% level.

Figure 8 gives data on the dry weights of plants produced in the nineteen cation treatments. Significant differences in dry

weights of stems and roots, as well as leaves produced, resulted from the treatment (table 3). As in the case of the September harvest, growth responses to the cation deficiencies were less well marked than were the responses to the anion deficiencies. Magnesium deficiency, however, was more severe in the March than in the September harvest. Figure 9 gives data on the resin and rubber concentrations found in the nineteen cation treatments. Significant differences

TABLE 4

IONIC COMPOSITIONS OF SOLUTIONS RESULTING IN MAXIMUM GROWTH AND RUBBER ACCUMULATION

Solution	Ionic concentration (me./l.)					
	NO_3	SO_4	H_2PO_4	Mg	K	Ca
2	19.8	2.8	2.8	9.0	4.5	12.0
4	17.0	0.0*	8.5	9.0	4.5	12.0
5	14.2	5.7	5.7	9.0	4.5	12.0
22	8.8	4.7	8.8	21.0	2.0	4.0
24	10.0	9.0	10.0	18.0	0.0†	12.0
25	8.7	4.8	8.7	15.0	4.0	8.0

* Maximum of 0.15 me./l. of SO_4 present as impurities, etc.
† Maximum of 0.15 me./l. of K present as impurities, etc.

in both quantities resulted from the treatments (table 3), although resin concentration varied by but 16% as between the largest and the smallest values. Rubber concentration was influenced in particular by magnesium concentration, and it decreased regularly as the concentration of this ion in the nutrient decreased. This result is not wholly in accord with those of the September harvest. In the earlier harvest, lowered rubber concentration appeared to result only with nutrients low in both potassium and magnesium, while the highest concentration was found in treatment 16, high in potassium but deficient in both magnesium and calcium. The results of the cation treatments show, as did those of the anion, that treatments advantageous for rubber accumulation early in the season may not maintain their superiority throughout the season.

Discussion of experiment i.—The plants of this experiment, although thrifty and vigorous in appearance, accumulated markedly less dry weight over the 8.5-month experimental period than did similar stock planted in the field. It has been repeatedly observed that guayule plants cultured in 1- to 2-gallon containers fail to make as rapid growth as similar plants in the field. This is not due to deficiency of any major nutrient, as can be seen from the results of the present experiment. Neither does it appear to be due to a deficiency of any micronutrient, since deficiencies for these were not obtainable under the conditions employed.

Maximum growth from a standpoint of dry-weight accumulation was produced in anion treatments 2, 4, and 5 and in cation treatments 22, 24, and 25. In these solutions (table 4) nitrate was present in relatively high concentration (9–20 me./l.). Phosphate was also present in moderate concentrations (2.8–10.0 me./l.), while sulphate varied from 9.0 to 0.15, the latter being the concentration present in solution 4 as impurities. The highest rubber concentrations (5.5%) were obtained in solutions 4 and 5. Rubber accumulation in treatments 4 and 5 compares favorably with the accumulation by fieldgrown plants during the first growing season. It is evident from the compositions of solutions 4 and 5 (table 4) that rubber accumulation—as well as growth—is relatively independent of sulphate concentration but markedly dependent on phosphate and nitrate concentration. Solution 2, which yielded good growth and high rubber concentration, approximates the nutrient

solution of HOAGLAND and ARNON (3) in composition.

Although growth, as measured by dry-weight accumulation, was as great in the optimal solutions of the cation series as in those of the anion series, rubber concentration was markedly less. But the maximum rubber concentrations attained in the cation series do correspond with those in the anion series at comparable nitrate levels. In a repetition of experiment I, it would be desirable to use the optimum anion concentrations in the composition of the cation solutions. Conversely, however, the concentrations of cations used in the composition of the anion solutions of experiment I would appear to have been near optimal. It should be noted that although treatment 9 is identical with treatment 29, still there are significant differences in growth and rubber concentrations as between plants grown under the two treatments. This reflects the fact already mentioned that the cation and anion treatments were set up as separate experiments in separate plots.

Experiment II

During the second week in June, 1942, three nursery seedlings of the same lot as used in experiment I were planted in each of approximately 200 1-gallon cans. The cans, which had been previously painted with nontoxic asphaltum paint and provided with drainage, were filled with 4-mesh gravel. On July 1, the plants were thinned to two per container, and 140 cultures selected for uniformity were chosen for the initiation of experiment II.

NUTRIENT SOLUTIONS.—The seven nutrient solutions used in experiment II supplied nitrogen either entirely as nitrate, entirely as ammonium, or as mixtures of the two. The solutions (table 5) were modified from those suggested by VICKERY *et al.* (4). K, PO_4, Ca, N, and Mg are present in the same concentrations in all seven solutions (table 7). Both Cl and SO_4 increase as the ratio of ammonium to nitrate increases. Total osmotic pressure of the ammonium-containing solutions is likewise greater than that of the solution containing only nitrate nitrogen. A preliminary experiment indicated that, over a 3-month growth period, variations in osmotic pressure

TABLE 5

CONCENTRATIONS OF INGREDIENTS OF NUTRIENT SOLUTIONS SUPPLYING NITROGEN AS NITRATE AND AMMONIUM AT DIFFERENT RATIOS (MILLIMOLS PER LITER)

SALT	SOLUTION NUMBER						
	1	2	3	4	5	6	7
	Percentage N as NO_3						
	100	80	60	40	20	10	0
KH_2PO_4	6.5	6.5	6.5	6.5	6.5	6.5	6.5
$Ca(NO_3)_2$	8.6	6.9	5.2	3.4	1.7	0.9	0.0
$CaCl_2$	0.0	1.7	3.4	5.2	6.9	7.7	8.6
$(NH_4)_2SO_4$	0.0	1.7	3.4	5.2	6.9	7.7	8.6
$MgSO_4$	2.1	2.1	2.1	2.1	2.1	2.1	2.1

of the nutrient between the limits of the solutions of table 7 exert only minor effects on growth. VICKERY *et al.* have also shown for tobacco that the influence of the added Cl and SO_4 concentrations is negligible in comparison with the influence of the ammonium ion itself.

Nutrient solution was applied at the rate of 300 cc. per culture three times a week. Tap water was further supplied as needed. All nutrient solutions were adjusted to pH 5.6 before use.

EXPERIMENTAL DESIGN.— The seven treatments of experiment II were tested in each of five plots. In each plot each treatment was represented by four cul-

tures, each of two plants. Thus each treatment was tested on forty plants. The data were treated by the analysis of variance.

HARVEST OF OCTOBER 1.—On October 1, after 3 months of treatment, twenty plants of each treatment were harvested, representing two plants in each of two cultures from each of the five plots. The plants were washed free of sand, dried rapidly at 60° C., weighed (the leaves separately), and the stem and root portions ground in a Wiley mill and analyzed for rubber and resin.

TABLE 6

INFLUENCE OF NUTRITION WITH VARYING RATIOS OF AMMONIUM TO NITRATE NITROGEN ON GROWTH AND RUBBER ACCUMULATION. HARVEST OF OCTOBER 1

Treatment no.	NO_3 (%)	NH_4 (%)	Dry wt. per 2 plants (gm.)		Resin‡ (%)	Rubber§ (%)
			Stems and roots*	Leaves†		
1	100	0	10.21	11.28	3.83	1.28
2	80	20	11.20	12.13	3.78	1.16
3	60	40	11.73	12.16	3.92	1.14
4	40	60	10.90	11.41	4.07	1.16
5	20	80	9.81	10.31	4.32	1.14
6	10	90	7.86	8.77	4.00	1.07
7	0	100	6.83	8.15	4.00	0.76

* Difference of 1.49 required for significance at 5% level; of 1.98 at 1% level.

† Difference of 1.61 required for significance at 5% level; of 2.14 at 1% level.

‡ Difference of 0.21 required for significance at 5% level; of 0.28 at 1% level.

§ Difference of 0.25 required for significance at 5% level; of 0.33 at 1% level.

Table 6 summarizes the values obtained for dry weight, percentage resin, and percentage rubber. Plants receiving 0, 20, 40, 60, or 80 % of their nitrogen as ammonium did not differ significantly as to dry weight of stem and root, dry weight of leaves, or percentage of rubber, while percentage of resin was but slightly affected. Plants which received 90% of their nitrogen as ammonium, however, were markedly reduced in dry weight and in rubber concentration as compared with plants receiving less ammonium ion. Still more marked reduction in dry weight and rubber concentration resulted from the application of 100% of the nitrogen as ammonium.

HARVEST OF MARCH 1.—During the late fall and winter months, the plants of all treatments maintained a dormant aspect. On March 1, after 8 months of treatment, the second harvest was made. Each treatment was again represented by two plants from each of two cultures from each of five plots, a total of twenty plants. The plants were dried, weighed, and analyzed as before. Table 7 gives a summary of the data. In experiment II, as in experiment I, marked increases in dry weight of stems and roots took place during the winter, while dry weight of leaves decreased owing to shedding of the older leaves. Treatments in which 0, 20, 40, or 60 % of the nitrogen was present as ammonium did not result in plants which differed significantly from one another as to dry weight of stems and roots, percentage of resin, or percentage of rubber. The plants which received 80, 90, or 100 % of their nitrogen as ammonium yielded progressively lower dry weights and rubber concentrations.

DISCUSSION OF EXPERIMENT II.—The results of experiment II parallel those of CLARK (1) with tomato and of VICKERY (4) with tobacco, in that growth as measured by accumulation of dry weight was decreased as the ratio of ammonium nitrogen to nitrate nitrogen in the medium was increased. Both CLARK and VICKERY have pointed out the great influence which the type of the available nitrogen exerts on the composition of the

plant. In the present experiment a significant reduction in rubber concentration was apparently brought about as a result of the presence of considerable amounts of ammonium ion in the nutrient solution, pointing again to the importance of the kind of available nitrogen in determining plant constitution. Significant reduction in rubber percentage, in both the October and the March harvests, resulted exclusively in treatments where dry weight accumulation was likewise significantly reduced as a result of high ammonium nitrate ratios. While the physiological significance of the apparent deleterious effect of ammonium ions on growth and rubber accumulation is not evident at present, still this experiment does indicate that the use of ammonium ions in the nutrition of guayule plants may be undesirable under some conditions.

TABLE 7

INFLUENCE OF NUTRITION WITH VARYING RATIOS OF AMMONIUM TO NITRATE NITROGEN ON GROWTH AND RUBBER ACCUMULATION. HARVEST OF MARCH 1

Treatment no.	NO_3 (%)	NH_4 (%)	Dry wt. per 2 plants (gm.)		Resin‡ (%)	Rubber§ (%)
			Stems and roots*	Leaves†		
1	100	0	17.71	5.98	4.54	4.46
2	80	20	18.75	6.78	4.78	4.64
3	60	40	17.42	5.35	4.56	4.37
4	40	60	18.54	4.29	4.64	4.62
5	20	80	14.95	2.77	4.49	4.36
6	10	90	12.71	2.69	4.42	3.24
7	0	100	10.83	1.66	4.08	2.51

* Difference of 3.11 required for significance at 5% level; of 4.13 at 1% level.

† Difference of 0.98 required for significance at 5% level; of 1.30 at 1% level.

‡ Difference of 0.24 required for significance at 5% level; of 0.32 at 1% level.

§ Difference of 0.51 required for significance at 5% level; of 0.68 at 1% level.

Summary

1. Guayule plants were grown outdoors in gravel culture over a period of 8.5 months and supplied with thirty-eight different nutrient solutions of varying anion and cation composition. Two harvests were made, one at the end of the summer growing season and one at the end of the winter—or principal rubber-accumulating season.

2. Growth and rubber accumulation were affected by nitrogen supply more than by the supply of any other major nutritional element under the conditions used. Plants which received little nitrogen and which showed reduced growth accumulated little rubber as compared with plants at higher nitrogen levels. The highest level of rubber accumulation was in plants which received 14–17 milliequivalents of nitrogen as nitrate per liter of nutrient solution. These plants contained 5.5% rubber, based on dry weight of the defoliated plant, at the winter harvest.

3. Growth and rubber accumulation were both diminished in phosphate-deficient plants as compared with plants which received abundant phosphate. No responses to sulphate deficiency were evident.

4. Growth and rubber accumulation were relatively independent of the concentrations of calcium and potassium in the nutrient solution, but both were depressed under conditions of low magnesium concentration.

5. Plants were supplied with nitrogen in the form of nitrate or of ammonium, or as mixtures of the two. Those which received most or all of their nitrogen as ammonium grew less and accumulated less rubber over an 8-month period than plants which received all or most of their nitrogen as nitrate.

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LITERATURE CITED

1. CLARK, H. E., Effect of ammonium and of nitrate nitrogen on the composition of the tomato plant. Plant Physiol. 11:5–24. 1936.
2. HAMNER, C. L., Growth responses of Biloxi soybeans to variation in relative concentrations of phosphate and nitrate in the nutrient solution. BOT. GAZ. 101:637–649. 1940.
3. HOAGLAND, D. R., and ARNON, D. I., The water culture method of growing plants without soil. Univ. Calif. Coll. Agr. Circ. 347. 1938.
4. VICKERY, W. B., PUCHER, G. W., WAKEMAN, A. S., and LEAVENWORTH, C. S., Chemical investigations of the tobacco plant. VIII. The effect on the composition of the plant of the form in which nitrogen is supplied. Conn. Agr. Exp. Sta. Bull. 442. 1940.

EXTENT OF AUXIN-PRECURSOR HYDROLYSIS IN DIFFERENT AVENA ASSAY METHODS

G. S. AVERY, JR., J. BERGER, AND B. SHALUCHA

Introduction

In a recent study of auxins (10) the statement was made that the "deseeded test responds not only to auxin but also to auxin precursor."[1] It was obviously important to test the validity of this view, as applied to both the deseeded and modified standard methods, since the deseeded *Avena* test has been used in this laboratory for the past 4 years. Our study shows that in neither test method is there a significant conversion of precursor to auxin.

When the deseeded test method was first described (9), it was pointed out that it was designed for the detection of precursors of auxin. SKOOG, however, showed that no curvatures were obtained from *Avena* auxin precursor in the deseeded test in 8 hours; after 15 hours he reported curvatures. WENT (10) concluded that the response of deseeded plants to auxin diffused from freshly excised *Avena* coleoptile segments is almost exclusively due to auxin precursor. If the duration of the test was less than 10 hours (and the actual time is not specified in the paper in question, although a private communication from Professor WENT states that the time was 5 hours), then this is in direct contradiction to SKOOG's findings. We wish to emphasize at this point that the deseeded test as used in our laboratory is of exactly 5 hours' duration; that is, from time of application of agar blocks to the time of photographing.

MATERIAL AND METHODS.—Three general types of material were used as sources of auxin precursor: (*a*) extracts of immature and mature (dormant) kernels of *Zea mays* prepared in various ways; (*b*) a zein preparation from maize kernels; and (*c*) purified auxin-precursor preparations from maize kernels. These materials were extracted or otherwise treated as indicated in the tables and in most instances were assayed by both the deseeded (1) and the modified standard (8) *Avena* test methods. All data are based on *Avena* curvatures in the proportionality range.

Investigation

When WENT's statement appeared to the effect that "the curvature in the deseeded test is almost exclusively due to auxin precursor . . . ." with certain auxin preparations, we reviewed some of the data then on hand from work in our

[1] The term "precursor" as used here indicates a compound which is physiologically inactive in the *Avena* test but becomes auxin (physiologically active) upon hydrolysis.

laboratory. These are presented in table 1. The examples were selected because of their low "free" auxin content and high precursor yield, as determined by auxin activity after alkaline hydrolysis. Even if it is assumed that the entire yields in untreated extracts represent precursor converted to auxin by deseeded plants during the 5-hour test, then 1 or 2% is the maximum possible conversion.

Further to test whether the response to unhydrolyzed maize extracts was due to free auxin or to auxin converted from the precursor during the test, aqueous extracts were purified by ether extraction to remove as much of the free auxin as possible. Aqueous extracts of ground whole kernels of sugar corn were made at natural pH, then adjusted to pH 4–5 and shaken with three changes of ethyl ether (25 ml. each). The ether extracts containing the auxin were discarded. The assays given in table 2 are on the water residue, which is high in precursor. Part of the residue was assayed without further treatment, and part after alkaline hydrolysis; that is, autoclaved 30 minutes in M/20 borate buffer, pH 9.6, then adjusted to pH 6 before assay. Results of assays on such purified extracts are given in table 2. The important fact to note is that in spite of the ether purification, 0.7–1.9% of the total auxin activity still appears as "free" auxin. The question whether this figure represents residual traces of free auxin, or small amounts of auxin produced by actual conversion of precursor during the assay, cannot be answered by these data; they justify no more than a repetition of the first observation that if conversion does occur, it is less than 1–2%.

Conclusive evidence could be obtained only from purer preparations of precursor. Data on such preparations are given in table 3. The zein sample was one that had been thoroughly extracted with ether; the precursor samples were

TABLE 1

MAXIMUM POSSIBLE HYDROLYSIS OF AUXIN PRECURSOR DURING DESEEDED AVENA TEST, AS SHOWN BY ASSAYS ON AQUEOUS EXTRACTS OF GROUND CORN KERNELS. DATA FOR SAMPLES 1 AND 5 PUBLISHED PREVIOUSLY (4); PEDIGREES, ETC., AS IN EARLIER PAPER

SAMPLE NO.	MATERIAL	YIELD OF AUXIN, IN MILLIONS OF TDC* OR TENS OF MICROGRAMS OF INDOLEACETIC ACID (PER GRAM DRY WEIGHT) OF CORN		MAXIMUM POSSIBLE PRECURSOR (%) CONVERTED TO AUXIN DURING TEST
		Untreated†	Alkali hydrolyzed‡	
1....	Inbred 77, 10 days after pollination	0.80	34.7	2.3
2....	Hybrid 677×C14 (F_2), 12 days after pollination	0.30	23.4	1.3
3....	Inbred 75, 20 days after pollination	1.60	92.8	1.7
4....	Inbred 580, 58 days after pollination	0.20	19.7	1.0
5....	Country Gentleman, mature kernels	0.20	10.0	2.0
6....	Hybrid 75×77, mature kernels	0.39	18.9	2.1

* TDC (total degrees curvature), usual units employed in this laboratory (2), are defined in terms of deseeded test and applicable only to it.

† Ground tissue extracted with water at about 25° C. for 15 minutes; suspensions centrifuged and clear centrifugate assayed for auxin activity.

‡ Ground tissue heated in M/20 borate buffer, pH 9.6, at 120° C. for 15 minutes; suspensions then adjusted to pH 6 and clear centrifugates assayed for auxin.

isolated from ground corn kernels in a manner described elsewhere (5). After alkaline conversion, preparation DE-5—for example—was 2.4% pure indoleacetic acid, calculated on a dry-weight basis. It may be seen from the data that with the zein, a maximum of 0.35%

TABLE 2

MAXIMUM POSSIBLE HYDROLYSIS OF AUXIN PRECURSOR DURING AVENA ASSAY AS SHOWN BY ASSAYS ON PARTIALLY PURIFIED AQUEOUS EXTRACTS OF SWEET CORN

Sample no.	Material	Treatment of ether-extracted water residue	Avena assays*				Maximum† possible precursor (%) converted to auxin during test	
			Deseeded		Modified standard			
			Dilution (ml.)	Curvature (°)	Dilution (ml.)	Curvature (°)	Deseeded	Modified standard
1......	Frozen sweet corn (Golden Bantam)	None	100	13.9	25	9.0	....	....
		Alkali hydrolyzed	8000	9.1	4000	8.2	1.9	0.7
2......	Frozen sweet corn (Golden Bantam)	None	200	10.2	25	9.7	....	....
		Alkali hydrolyzed	8000	12.9	4000	9.1	2.0	0.7
3......	Fresh corn (Early Yellow)	None	200	9.4	50	10.8	....	....
		Alkali hydrolyzed	8000	9.1	4000	10.5	2.6	1.3
4......	Fresh corn (Early Yellow)	None	200	13.1	50	20.8	....	....
		Alkali hydrolyzed	8000	11.7	8000	7.1	2.8	1.8

* Controls as follows: Deseeded test, 10 micrograms indoleacetic acid per liter, 9.2° curvature; modified standard test, 20 micrograms indoleacetic acid per liter, 8.2° curvature.

† Maximum percentage hydrolysis: Assuming that all the yield in the untreated preparation is due to hydrolysis of the pre cursor by the *Avena* coleoptile, then, for sample no. 1, deseeded method, the conversion is $\frac{13.9}{9.1} \times \frac{100}{8000} \times 100 = 1.9$ %.

TABLE 3

MAXIMUM POSSIBLE HYDROLYSIS OF AUXIN PRECURSOR DURING AVENA TEST AS SHOWN BY ASSAYS ON ZEIN AND PURIFIED CORN AUXIN PRECURSOR

Sample	Treatment	Avena assays				Precursor (%) apparently converted to auxin during test	
		Deseeded		Modified standard			
		Dilution‡ (ml.)	Curvature in degrees	Dilution‡ (ml.)	Curvature in degrees	Deseeded	Modified standard
Zein*	None.............	75	10.9	37.5	7.5	....	....
	Alkali hydrolyzed...	30,000	7.7	15,000	8.5	0.35	0.22
Purified auxin precursor DE-5†	None.............	250	13.4	125	10.0	....	....
	Alkali hydrolyzed...	1,600,000	14.8	400,000	17.5	0.014	0.018

* Zein preparation extracted for 3 days in ether by Dr. S. A. GORDON, to whom we are indebted for the material.

† Precursor preparation of 2.4% purity.

‡ Calculated to basis of 1 gm. of sample tested.

conversion was obtained in the deseeded assay, as against 0.22% in the modified standard test. With precursor DE-5 the extremely small "apparent" conversion value of 0.014% was obtained in the deseeded test. Approximately the same value was obtained in the modified standard test. Other precursor concentrates gave apparent conversions of equal magnitude in both tests. With increasing purification of precursor, the conversion value decreased. Thus it is highly probable that even the small curvatures produced by unhydrolyzed precursor concentrates are due to contaminating amounts of free auxin and not to precursor conversion at all. Precursor preparation DE-5 is typical of several isolations made from corn. Since these purified concentrates contain as high as 50% of the precursor present in corn, it may be assumed that similar resistance to conversion exists for the major portion of the auxin precursor in corn. In the light of these results, the apparent conversion values in tables 1 and 2 may be interpreted as representing free auxin and not real conversion.

It is obvious that if there is any conversion of maize auxin precursor to auxin in either test method, it is so small as to be of no consequence in assays of natural extracts.

Discussion

The evidence presented here supports a portion of SKOOG's work and may be briefly summarized as follows: curvatures obtained during the 5-hour test period from untreated aqueous extracts of corn represent free auxin, and not auxin converted from a precursor. The evidence presented is equally applicable, whether maize extracts contain one or more than one auxin precursor.

As to the non-hydrolyzability during the deseeded test of auxin precursors other than that from corn, it may be suggested that auxin precursors from all the cereal grains will probably respond in a similar manner. Precursors have been demonstrated by the deseeded method in maize, wheat, oats, rice, and even in turnip and beet seeds by assays on untreated and alkali hydrolyzed preparations (2). The detection of precursors in these materials rests clearly on alkaline hydrolysis of the preparation before assay and not upon conversion by the test plant.

The response of deseeded test plants to a variety of synthetic compounds of a precursor nature has already been studied: naphthalene and indole acetamides, methyl naphthaleneacetate (3), indole ethylamine and tryptophan (9), all show no activity in a 5-hour deseeded test. After chemical hydrolysis, however, these compounds produce *Avena* curvatures.

It seems more or less unfortunate that the impression has crept into the literature (10, 11) that deseeded test plants respond to auxin precursor in 2–6 hours. This is not in agreement with the experimental data presented by SKOOG, although it is apparently upon his work that the impression is based. Figure 29 in (11) contains no curve to substantiate the statement in the text that agar blocks containing auxin precursor, if left on the deseeded test plants 2–6 hours, give rise to distinct auxin curvatures. SKOOG's original curve (9, curve 2, fig. 8) shows no measurable curvature from precursor until 15 hours.

In our experience the same figure 29 (11) gives a misleading impression as regards the response of deseeded test plants to pure indoleacetic acid. The response in the 5-hour deseeded test is strictly a proportional one (as related to time and concentration) when the concentration

is between 0 and 20 micrograms per liter (fig. 1). The odd shape of curve A in figure 29 (11) is difficult to understand, since a 100-microgram per liter solution of indoleacetic acid gives the response shown in figure 1 of this paper; our test even at this relatively high concentration also shows proportional response up to

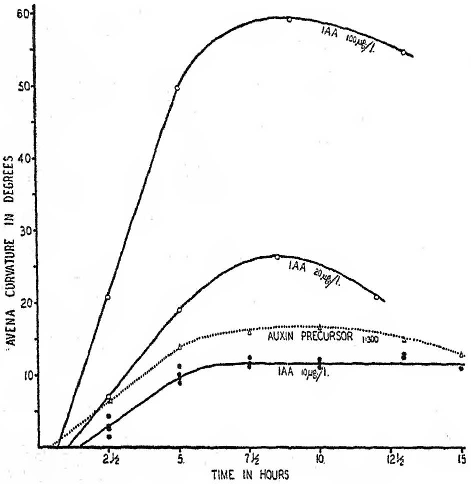

FIG. 1.—Coleoptile curvatures in *Avena* test plants over 15-hour test period (deseeded method). Indoleacetic acid (IAA) in 1.5% agar, tested at concentrations of 10, 20, and 100 micrograms per liter; partially purified maize auxin precursor (5) tested at 1:300 dilution. For IAA curves, each point is average of 2 dozen test plants (one day's run); for auxin precursor preparation, each point on curve is average of 4 dozen test plants (one day's run). Darkroom maintained at 88–90% relative humidity, 25° C., and lighted only with phototropically inactive light.

5 hours. (We prefer to work with curvatures of less than 20 degrees.)

The slope of the curve for the auxin precursor is in close agreement with those produced by 10 micrograms per liter solutions of indoleacetic acid (fig. 1); the *Avena* curvatures are undoubtedly due to a small amount of free auxin in the precursor preparation. This fact can be regarded only as supporting the other evidence as to lack of precursor conversion in the deseeded test plant. If there were conversion of the precursor into auxin, a steeper slope would be expected in the curve as well as a continuing rise after $7\frac{1}{2}$ hours.

Does the deseeded test plant indeed convert auxin precursor(s) into auxin, even after 15 hours? In the light of the work by GORDON and WILDMAN (6), who show that a small amount of auxin is produced when tryptophan is heated gently (65° C.) for 1 minute with agar, part of SKOOG's original experiments might be explained as follows: In the preparation of the tryptophan in agar blocks, the short period of heating resulted in the formation of a very small amount of auxin, which, over a long period of time, gave curvatures of small magnitude (SKOOG reported approximately 5 degrees). Or, if there was no conversion of tryptophan into auxin during the preparation of agar blocks, it is equally possible that such a conversion occurred in the blocks at darkroom temperatures in the course of the 15-hour period they were on the test plants. In neither case would the curvatures be the result of precursor entering the test plants as such, and there being converted into auxin.

The curvature obtained with *Avena* precursor extracts might similarly have been due to a response of the deseeded plant to a very small amount of free auxin, and not to precursor conversion. The fact that the deseeded test continues for 5 hours enables it to detect much smaller amounts of auxin than are detectable by the original or modified standard methods ($1\frac{1}{2}$ hours).

Bearing indirectly on this problem is VAN OVERBEEK'S (7) work on "exhaustive diffusion" and "exhaustive extractions," from which he concludes that decapitated coleoptile tips (of maize and oats) standing on agar for periods of

many hours slowly convert precursor into auxin, and in the latter form deliver it to the agar by diffusion. This apparently means that excised coleoptile tissues can convert precursor into auxin; it does not indicate that the test plant is in any way concerned in converting precursor to auxin.

There is as yet no certain evidence that the coleoptiles of test plants respond to auxin precursor as such, in agar, and convert it into auxin, ultimately producing curvature in the *Avena* test (whether it be the deseeded test or some other).

Summary

1. It has been stated in the literature that deseeded *Avena* test plants respond to auxin precursor in 2–6 hours. The evidence presented here, as a result of studies on crude and purified preparations of a maize auxin precursor, shows that curvatures obtained during the 5-hour test period represent free auxin, and not auxin converted from a precursor.

2. The question is raised whether under any conditions deseeded test plants ever convert precursor into auxin, even if employed for long test periods.

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LITERATURE CITED

1. AVERY, G. S., JR., CREIGHTON, H. B., and HOCK, C., A low cost chamber for phytohormone tests. Amer. Jour. Bot. 26:360–365. 1939.
2. AVERY, G. S., JR., BERGER, J., and SHALUCHA, B., The total extraction of free auxin and auxin precursor from plant tissue. Amer. Jour. Bot. 28:596–607. 1941.
3. AVERY, G. S., JR., BERGER, J., and SHALUCHA, B., Comparative activity of synthetic auxins and derivatives. BOT. GAZ. 104:281–287. 1942.
4. AVERY, G. S., JR., BERGER, J., and SHALUCHA, B., Auxin content of maize kernels during ontogeny, from plants of varying heterotic vigor. Amer. Jour. Bot. 29:765–772. 1942.
5. BERGER, J., and AVERY, G. S., JR., Isolation of an auxin precursor and an auxin (indoleacetic acid) from maize. Amer. Jour. Bot. (in press) 1944.
6. GORDON, S. A., and WILDMAN, S. G., The conversion of tryptophane to a plant growth substance by conditions of mild alkalinity. Jour. Biol. Chem. 147:389–398. 1943.
7. OVERBEEK, J. VAN, A quantitative study of auxin and its precursor in coleoptiles. Amer. Jour. Bot. 28:1–10. 1941.
8. SCHNEIDER, C. L., and WENT, F. W., A photokymograph for the analysis of the *Avena* test. BOT. GAZ. 99:470–496. 1938.
9. SKOOG, F., A deseeded *Avena* test method for small amounts of auxin and auxin precursors. Jour. Gen. Physiol. 20:311–334. 1937.
10. WENT, F. W., Growth, auxin, and tropisms in decapitated *Avena* coleoptiles. Plant Physiol. 17:236–249. 1942.
11. WENT, F. W., and THIMANN, K. V., Phytohormones. New York. 1937.

APOSPORY AND APOGAMY IN A SPECIES OF TECTARIA

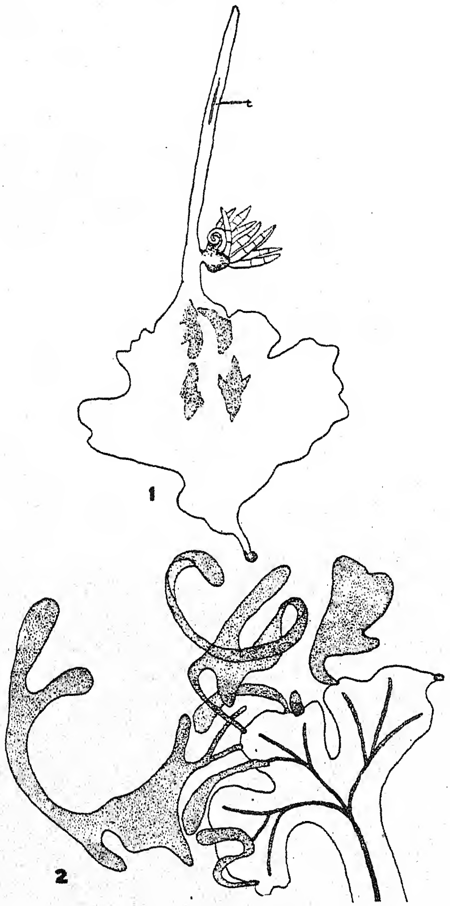
W. N. STEIL

Spores of a *Tectaria* species, labeled *Dryopteris trifoliata* but undoubtedly *Tectaria trifoliata*, obtained from the Botanical Garden of Leiden, Holland, April, 1935, were sown on the surface of a Beyernick's solution, as modified by MOORE (5), and which had first been put into Erlenmeyer flasks and sterilized in an autoclave. The observations on the gametophyte of this fern in the first cultures extended over a period of a year and were confirmed by two other sets of cultures, the spores of which were later obtained from the same source and sown September, 1935, and June, 1939.

Observations

GAMETOPHYTE.—In most respects the gametophyte resembles that of a *Dryopteris* species in its heart-shaped form and

large size. There is a tendency to lobing, however, even when it is grown under favorable light conditions. The hairs, so characteristic of the gametophyte of *Dryopteris*, are absent. Archegonia are never produced, but antheridia with normal antherozoids are formed in considerable numbers.



FIGS. 1, 2.—*Tectaria* species: Fig. 1, variegated gametophyte, shaded portions representing darker chlorophyllose regions. Tonguelike outgrowth with tracheids (*t*) bears young apogamous embryo. Fig. 2, apogamously produced sporophyte with gametophytes of aposporous origin.

The majority of the gametophytes were characterized by peculiar regions, varying from nearly colorless to dark green cells. The cells of the nearly white portions contained fewer and paler plastids, some of them being colorless. The thickness of the walls of the cells varied with their color, the greenest ones possessing the thickest walls. Frequently the nearly albinic cells were considerably smaller than the ordinary chlorophyllose cells. A large gametophyte with four dark green regions is shown in figure 1. Such regions were also observed in the gametophytes of aposporous origin. Similar gametophytes of several other *Tectaria* species and of a *Dryopteris* species have also been observed. Earlier, fern gametophytes of this nature have been described by ANDERSSON (1) and ANDERSSON-KOTTÖ (2, 3).

APOGAMOUS EMBRYO.—The embryo is of apogamous origin. The first evidence of such an embryo, as has been found in other apogamous ferns (4, 6, 7, 9, 10), is the appearance of tracheids in a less chlorophyllose region just back of the apical notch of the gametophyte.

In the sinus of the prothallium a lobe is always produced, from which a flat, tonguelike, cylindrical, or conical process may be formed and in which tracheids may also appear (fig. 1). On some portions of the projection the apogamous sporophyte originates. The embryo develops in the manner first described by FARLOW (4) in *Pteris cretica* var. *albolineata*, namely, in order of parts: leaf, root, and stem (the foot being absent).

At the base of the embryo (fig. 1), and from the petiole and blade of the young sporophyte, hairs—each consisting of several cells—are produced in large numbers. The hair possesses a sharp-pointed terminal cell (fig. 4) which bears fewer chloroplasts than the others, and hence is often almost colorless.

APOSPOROUS GAMETOPHYTE.—This originates in several ways (figs. 2–4), the

most common one being from one or more cells of a hair of the apogamously produced sporophyte. The nearly colorless terminal hair cell is apparently never involved in the formation of a gametophyte.

The first visible evidence of the origin of a gametophyte from a hair is the multiplication of the number of chloroplasts in its cells (figs. 4, 5). The gametophytes of this nature, originating from the hairs at the base of the embryo, are produced in great numbers, and sometimes several are formed from the hairs of a single petiole of the first leaf (fig. 6). No gametophytes were observed to develop from the hairs of the blade of the leaf of the sporophyte.

The gametophyte originates from any cells of the hair except the terminal one (figs. 4–9), as already stated. Only once was a rhizoid observed to be formed (fig. 8) from a gametophyte of aposporous origin. Only early stages in the development of the gametophyte were studied, but it can be assumed that the later stages are in all respects similar to those originating from a spore.

Of less frequent occurrence are the gametophytes originating either from marginal epidermal cells or from epidermal cells near the margin of the first leaf of the sporophyte (fig. 2). As is shown in the figure, a considerable number of gametophytes of this nature may be produced.

The most interesting origin of the gametophyte is illustrated in figure 3, showing the terminal portion of the leaf blade. A lobe, similar to that shown at the left, was also present at the right but was injured in mounting. A large part of the end of the leaf is distinctly gametophytic. This portion has produced plates of cells which in turn formed smaller plates and filaments. The conspicuous veins extend into regions distinctly gametophytic.

A number of "lighter" regions, including some which are albinic, can be observed in different portions of the leaf as well as in the terminal gametophytic outgrowths. A small terminal portion of another similar leaf is shown in figure 10.

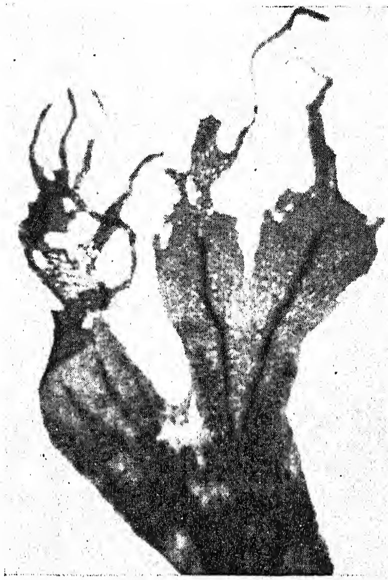


FIG. 3.—Aposporous gametophytes produced by first leaf of sporophyte of apogamous origin.

The longer narrower cells represent those near the sinus of the modified leaf. The lighter areas could be readily observed with the low power of the microscope.

The writer (8, 10) has suggested the nature of the origin of apospory of the type just described by assuming, on account of the intimate connection between the sporophyte of apogamous origin and its gametophyte, that some of the cells may be carried upward by the developing leaf and, retaining their power to divide, may form ordinary gametophytes.

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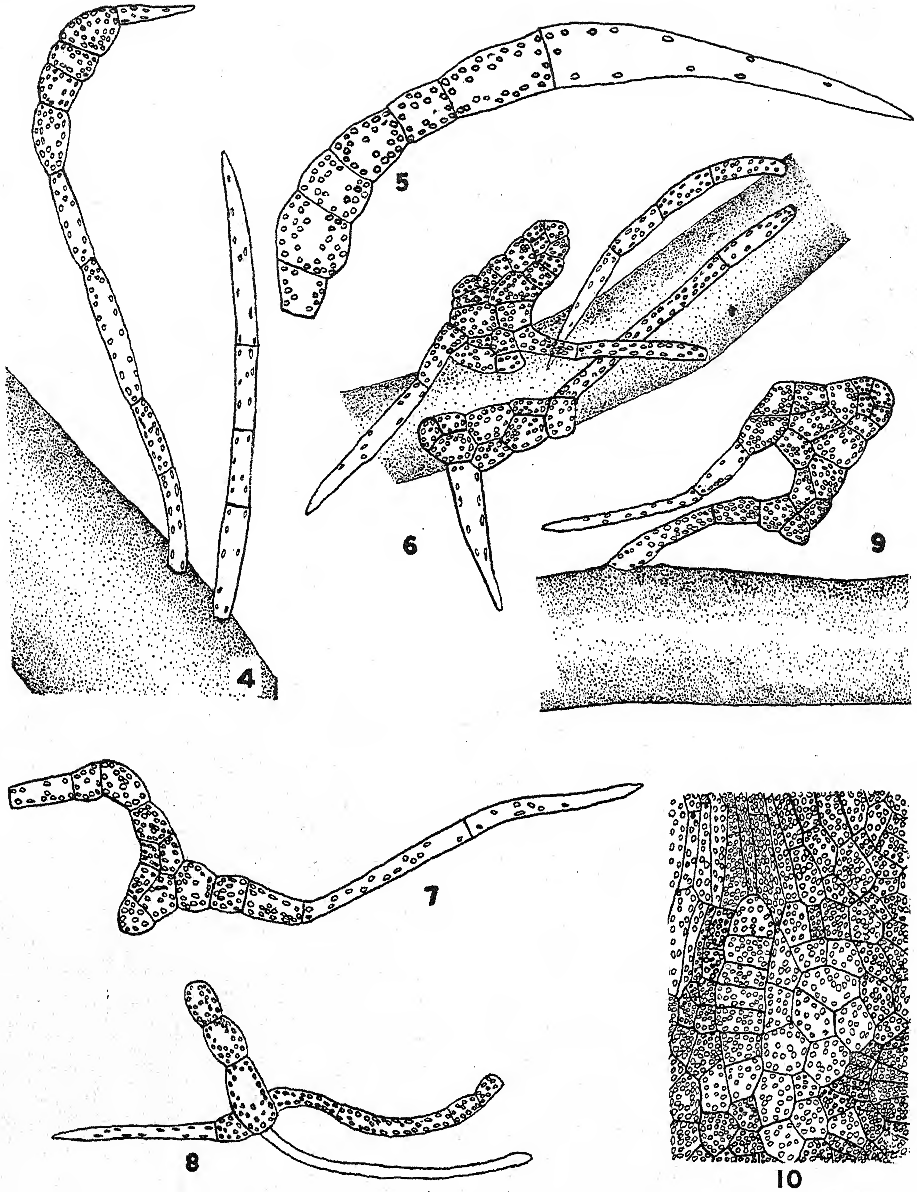

FIGS. 4–10.—*Tectaria* species. Fig. 4, beginning of aposporous gametophyte from one or more cells of one of the two hairs borne by petiole of leaf. Fig. 5, hair produced by petiole; six of the seven cells have become gametophyte. Fig. 6, two young prothallia produced aposporously from hairs of petiole of leaf. Each gametophyte possesses an apical cell. Fig. 7, young aposporously produced gametophyte removed from petiole of leaf of sporophyte. One basal cell and two at opposite end not involved in formation of gametophyte. Fig. 8, young gametophyte produced from only one cell of a hair; rhizoid also formed. Fig. 9, gametophyte produced by cells of a hair. Basal and terminal portion of hair have become nearly parallel in development of gametophyte, which shows well-defined apical cell. Fig. 10, portion of lamina of young leaf of sporophyte. Clearly defined lighter region and some of adjoining portions composed of cells distinctly gametophytic in character.

LITERATURE CITED

1. ANDERSSON, IRMA, The genetics of variegation in a fern. Jour. Genet. 13:1–11. 1927.
2. ANDERSON-KOTTÖ, IRMA, Variegation in three species of ferms (*Polystichum angulare*, *Lastrea strata*, and *Scolopendrium vulgare*). Leits. Ind. Abst. Vererb. 56:115–201. 1930.
3. ———, The genetics of ferns. Bibl. Genet. 8:269–294. 1931.
4. FARLOW, W. G., An asexual growth from the prothallium of *Pteris cretica*. Quart. Jour. Micr. Sci. 14:226–273. 1874.
5. MOORE, G.T., Methods for growing pure cultures of algae. Jour. Appl. Micros. 6:2309–2314. 1903.
6. STEIL, W. N., Studies in some new cases of apogamy in ferns. Bull. Torr. Bot. Club 45:93–108. 1918.
7. ———, A study of apogamy in *Nephrodium hirtipes*. Ann. Bot. 33:109–132. 1919.
8. ———, Vegetative reproduction and aposporous growths from the young sporophyte of *Polypodium iriodes*. Bull. Torr. Bot. Club 48:203–205. 1921.
9. ———, New cases of apogamy in certain homosporous leptosporangiate ferns. BOT. GAZ. 95: 164–167. 1933.
10. ———, Apogamy, apospory, and parthenogenesis in the pteridophytes. Bot. Rev. 5:433–453. 1939.

PECULIAR ANTHEROZOIDS OF A SPECIES OF CHARA

W. N. STEIL

In August, 1935, a species of *Chara* was found about 50 miles north of Milwaukee in a pool fed by an artesian well. Some of the plants were brought into the laboratory, where attempts were made to obtain the motile antherozoids. Collections of this species were made by Mr. KARL MILLER and the writer for several years from the pool and from a number of other places near Milwaukee. It was always found growing in cold water, often in a running stream.

All efforts to obtain the motile antherozoids failed until the alga was grown in the University greenhouse in the summer of 1937, when the temperature reached 100° F. for a considerable portion of the day. Under similar temperature conditions, for the past 6 years antherozoids were readily obtained in great numbers.

The technique for obtaining the antherozoids and for preparation of the slides has already been described (9). This species when grown in the laboratory frequently showed many cells of the spermatogenous filaments to be multinucleate. The nuclei were sometimes unequal in size but considerably smaller

FIGS. 1, 2.—Fig. 1, antherozoid of a *Chara* species with double vescicle. Fig. 2, antherozoid with three cilia.

than those in the ordinary spermatogenous cells. In a study of the development of the antherozoid, disturbances in the mitotic process occurred, similar to those described as incomplete mitosis by STEIL (3–8), and more recently as interrupted divisions by a number of investigators, including BLAKESLEE and AVERY (1) and NEBEL and RUTTLE (2). Small cells were also formed in the spermatogenous filaments. In some instances the walls of these smaller cells were oblique to the length of the filament.

Many cells of the filaments aborted, and hence antherozoids were not produced. Undoubtedly disturbances in the nuclear and cell division caused the irregularities in the cells and also their abortion.

Two unusual features were observed occasionally in the mature antherozoids. One of these is the double vesicle. An antherozoid (fig. 1) with a vesicle of this nature was fixed a short time after it had assumed its swimming motions, as is shown by the fact that the vesicle remained intact. No antherozoids with double anterior ends were observed. Another unusual feature is associated with the number of cilia, three or four being present (fig. 2). The ciliary band, partially removed from the anterior end of the body on which it is superimposed, shows the origin of the cilia, two of which are equal in length. The other cilium is of considerably greater length. Such peculiar antherozoids apparently have not heretofore been reported.

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LITERATURE CITED

1. BLAKESLEE, A. F., and AVERY, A. G., Methods of inducing doubling of chromosomes in plants by treatment with colchicine. Jour. Hered. **28**:393–411. 1937.
2. NEBEL, B. R., and RUTTLE, M. L., The cytological and genetical significance of colchicine. Jour. Hered. **29**:3–9. 1938.
3. STEIL, W. N., A study of apogamy in *Nephrodium hirtipes*. Ann. Bot. **33**:109–132. 1919.
4. ———, Nuclear and cell phenomena in the tapetum of *Botrychium virginianum*. Abstract, Bot. Soc. Amer. 1930.
5. ———, Some phenomena in ferns. Abstract, Bot. Soc. Amer. 1931.
6. ———, Incomplete nuclear divisions, not amitosis in the tapetum of some homosporous eusporangiate ferns. Science **77**:606–607. 1933.
7. ———, Incomplete nuclear and cell division in the tapetum of *Botrychium virginianum* and *Ophioglossum vulgatum*. Amer. Jour. Bot. **22**:409–425. 1935.
8. ———, Apogamy, apospory, and parthenogenesis in the pteridophytes. Bot. Rev. **5**:433–453. 1939.
9. ———, Antherozoids of some of the Characeae. BOT. GAZ. **102**:623–628. 1941.

CYTOGENETIC STUDIES ON TALINUM AND PORTULACA

ERICH STEINER

Introduction

Members of the Portulacaceae have received relatively little attention from cytologists. SUGIURA (7, 8, 9) found n = 9 in *Portulaca pusilla*, n = 26 in *P. oleracea* var. *sativa*, and n = 18 in *P. marginata*. *P. grandiflora* has been reported to have n = 9 by TJEBBES (11), OKURA (4), and BLACKBURN (see TISCHLER, 10). HAGERUP (2) found two varieties of *P. oleracea*, one with a haploid number of 9, and *P. oleracea gigas* with n = 27. COOPER (1) found n = 27 in *P. oleracea*.

Two species of *Talinum* have been counted, *T. patens* (n = 12) by BLACKBURN (10) and SUGIURA (7), and *T. purpureum* (n = 12) by SUGIURA (7).

Before the outbreak of the present war a cytotaxonomic study of *Portulaca* and *Talinum*, as well as other members of the family, was begun. Since the study is now interrupted, this paper is only a preliminary report and is confined to the preceding more easily grown genera.

MATERIAL AND METHODS.—As cytological material, these genera are unfavorable. Their succulent nature prohibits the use of leaf smears. Root tips are usually scarce. A suitable method for obtaining ample root-tip material consists in removal of the plant from the soil and allowing the roots to dry for a few hours. After drying, the plants are potted in sand, watered well, and placed in any situation where the transpiration rate is relatively high. Abundant root tips are produced in 12–24 hours.

The murky, heavily staining character of the cytoplasm also does not allow adequate chromosomal preparations with some of the usual techniques. Acetocarmine root-tip smears were impractical, partly because of the tendency for the cytoplasm to stain but primarily because the cells are so shaped as to make it impossible to obtain polar views of the metaphase plate. Furthermore, divisions are infrequent. The use of sectioned material is therefore more practical.

The following procedure was found effective in avoiding preparations with heavily stained cytoplasm. All material was fixed in Belling's modification of Navashin's fluid, and after dehydration and imbedding according to LA COUR'S (3) schedule, was sectioned at 10 μ. Newton's crystal-violet-iodine staining schedule was used, except that a 45-minute hydrolysis in 10% HCl at 60° C. was introduced just prior to staining. After hydrolysis, it was washed in running water for 10–15 minutes and subsequently stained. Destaining was carried out rapidly, less the stain be removed entirely. This method brings out the chromosomes in sharp contrast to a light background. An additional advantage lies in the fact that in some cases, where a chromosome plate is not quite clear, it may be flattened and spread somewhat, as is permitted by WARMKE'S (12) section-smear method. Drawings were made with camera lucida at table level.

Many of the species of *Talinum* and *Portulaca* were obtained through the courtesy of Dr. J. T. BALDWIN of the University of Michigan. Acknowledgments are also due Dr. O. E. WHITE of the Blandy Experimental Farm and Dr. V. L. CORY of the Texas Agricultural Experiment Station for collections. In most cases the original species descriptions were used to verify the species identification made by the collector.

Results

The chromosome numbers are listed in tables 1 and 2. Figures 1–16 show root-tip chromosomes of these species. All species of *Talinum* which were counted fit into a polyploid series, from a haploid number of 12 to one of 36. In the cortical area of the root tips of *T. parviflorum* are polyploid cells showing 96 chromosomes. Such cells are frequent here but were not found in other species.

The chromosomes of *Portulaca* show much greater variation among the species examined than do those of *Talinum*. The chromosomes of the various species differ markedly in size; they are also generally larger than those of *Talinum*. The species of *Portulaca* examined cannot be classi-

fied into a regular polyploid series as in *Talinum*.

There is no record of any interspecific hybridization in either genera, although *T. parviflorum* and *T. teretifolium* and twenty-five attempts to cross *T. aurantiacum* and *T. parviflorum* were unsuccessful.

TABLE 1

Species	Source	Diploid no.	Previous count	Investigator
Talinum				
appalachianum Wolf	Eight-mile Creek, St. Bernard, Ala.	24	....	....
parviflorum Nutt.	Springdale, Ark.	48	....	....
paniculatum Gaertn.	Pearce Seed Co., Moorestown, N.J.	24	24*	Blackburn (1934), Sugiura (1936)
paniculatum Gaertn.	Montevideo Botanical Gardens, Montevideo, Uruguay	24	....	....
paniculatum Gaertn.	Coimbra Botanical Gardens, Coimbra, Portugal	24	....	....
mengesii Wolf	St. Bernard, Ala.	24	....	....
teretifolium Pursh	Wake Forest, N.C.	48	....	....
teretifolium Pursh	Stone Mountain, Ga.	48	....	....
teretifolium Pursh	Bibb County, Ala.	24	....	....
aurantiacum Engelm.	Sonora, Tex.	48	....	....
variegata	Palmdale, Fla.	72	....	....
triangulare Wild	Coconut Grove, Fla.	72	....	....
species	Hidalgo County, Tex.	24	....	....

* Previous count of *T. paniculatum* reported in literature as a count of *T. patens*. These two species are, however, considered synonymous.

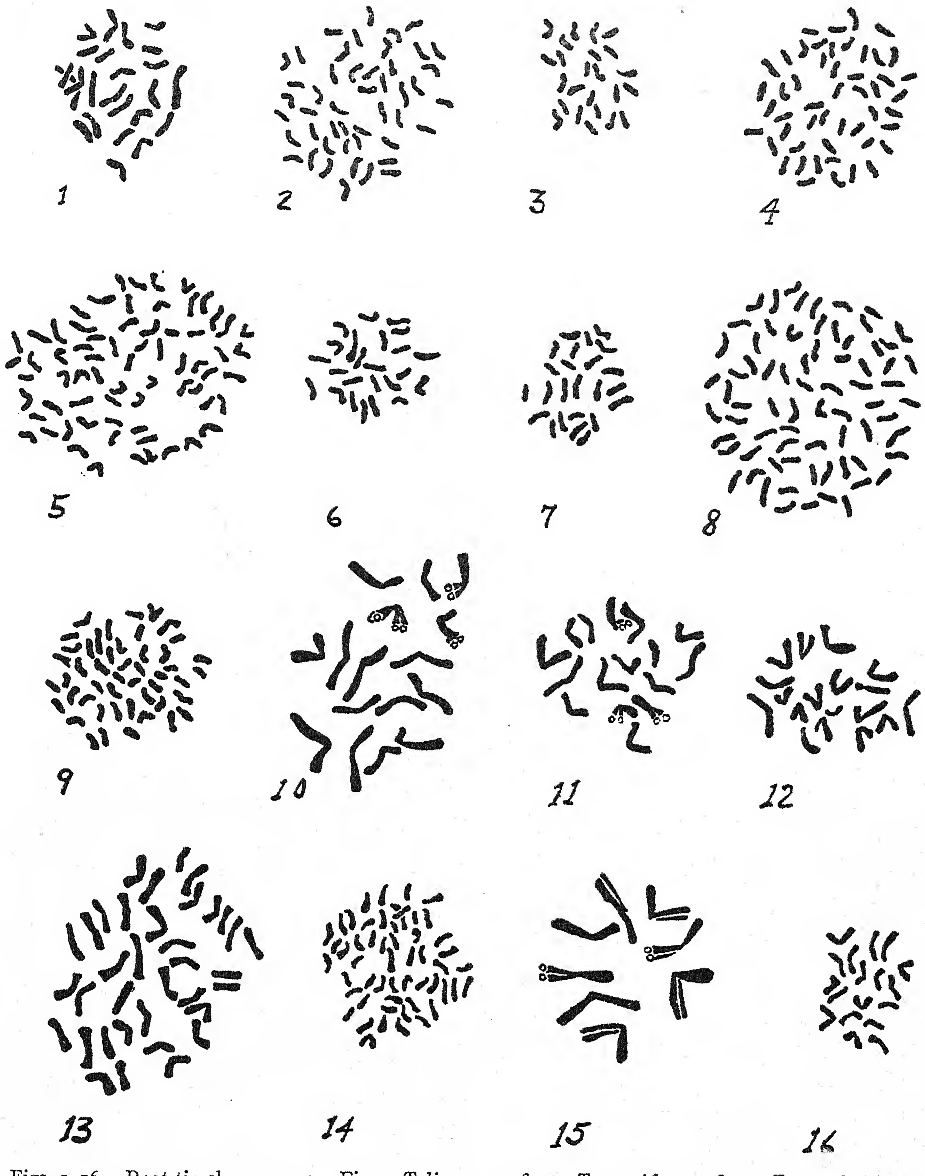
TABLE 2

Species	Source	Diploid no.	Previous count	Investigator
Portulaca				
grandiflora Hook.	Wood Seed Co., Richmond, Va.	18	18	Tjebbes (1928), Okura (1933), Blackburn (1934)
oleracea L.	Blandy Experimental Farm, Boyce, Va.	54	54	Cooper (1935)
pusilla	Royal Botanical Gardens, Stockholm, Sweden	18	18	Sugiura (1936)
pilosa L.	Daytona Beach, Fla.	16	....	....
smallii Wils.	Wake Forest, N.C.	16	....	....
marginata HBK.	Pearce Seed Co., Moorestown, N.J.	36	36	Sugiura (1936)
species	Springdale, Ark.	8	....	....

Portulaca grandiflora has been considerably used for genetical work. Crosses between some of the species were attempted. Fifty crosses between *P. grandiflora* and *P. marginata* were made, twenty-five using the latter as the female parent and twenty-five the reciprocal. No seed was set. Fifty attempts to cross

Discussion

The genus *Talinum* was first established by ADANSON in 1763. In the monograph of the genus by VON POELLNITZ (5), forty-seven species are recognized. Most of the species are distributed throughout the southwestern United States and Mexico, with a few



Figs. 1–16.—Root-tip chromosomes: Fig. 1, *Talinum* sp.; fig. 2, *T. parviflorum*; fig. 3, *T. appalachianum;* fig. 4, *T. teretifolium;* fig. 5, *T. triangulare;* fig. 6, *T. paniculatum;* fig. 7, *T. mengesii;* fig. 8, *T. variegata;* fig. 9, *T. aurantiacum;* fig. 10, *Portulaca smallii;* fig. 11, *P. pilosa;* fig. 12, *P. grandiflora;* fig. 13, *P. marginata;* fig. 14, *P. oleracea;* fig. 15, *Portulaca* sp.; fig. 16, *P. pusilla.* Figs. 1, 15, $\times$3200; others, $\times$2400.

species extending farther north and east. A few species are also found throughout Africa and Australia, but the genus is typically American.

The group may be divided into those forms with terete leaves, those with broad leaves, and an intermediate category with linear leaves. Too few species have been examined to determine conclusively whether there is any tendency toward a correlation of chromosome number and these taxonomic divisions of the genus. In all likelihood there is none, since such species as *T. paniculatum* and *T. triangulare*, two of the broad-leaved forms, are closely related yet exhibit chromosome number differences of a greater degree than the differences between these and terete-leaved species. The chromosome numbers of this genus have apparently little value in determining the eventual taxonomic position of the species; that must be based more or less completely upon morphological criteria.

The species listed in table 1 as *T. variegata* is not recognized by von Poellnitz, nor has it been possible to find any taxonomic description elsewhere. Horticulturally this plant is known either as *T. variegata* or as a variety of *T. paniculatum*. The plant differs from *T. paniculatum*, however, not only by its variegation but also by the thicker, more succulent leaves and the less vigorous, more open habit of growth. The chromosome number is also threefold that of *T. paniculatum*. These differences seem to warrant description of the plant as a distinct species.

The genus *Portulaca* has also been monographed by von Poellnitz (6), who recognizes 104 species. These are distributed throughout the world, but their greatest concentration occurs in South America. Far too few species have been examined cytologically to warrant definite conclusions, but some general considerations may be indicated. The Portulacas may be divided into three groups, characterized by broad, lanceolate, or terete leaves. The two broad-leaved species which have been counted have a higher chromosome number than those of the other two groups. Also, the numbers indicate that the evolution of the genus has involved chromosomal mutations—such as duplications and deficiencies—concurrent with polyploidy, as contrasted with *Talinum*, the evolution of which probably involved only simple polyploidy.

Two species, a *Talinum* from Hidalgo County, Texas, and a *Portulaca* from Springdale, Arkansas, have not been identified. The *Talinum* is a broad-leaved form related to *T. paniculatum*. The *Portulaca* belongs to the terete-leaved group. A thorough search of the literature has failed to reveal a species description which adequately fits in either case. It would seem, therefore, that both plants are new species. It is hoped that a description can be published in the future.

Attention is particularly called to this *Portulaca* from Springdale, Arkansas. This species might well be adapted as material for class study or in experimental work where a species with a small number of chromosomes morphologically distinguishable is desired.

Appreciation is expressed for guidance and constructive criticism rendered by Dr. O. E. White and Dr. Ladley Husted of the University of Virginia during the progress of this work.

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LITERATURE CITED

1. COOPER, D. C., Microsporogenesis and the development of the male gametes in *Portulaca oleracea*. Amer. Jour. Bot. **22**: 453–457. 1935.
2. HAGERUP, O., Über Polyploidie in Beziehung zu Klima, Ökologie, und Phylogenie. Hereditas **16**: 19–40. 1932.
3. LA COUR, L., Improvements in plant cytological technique. Bot. Rev. **3**: 241–258. 1937.
4. OKURA, E., A haploid plant in *Portulaca grandiflora* Hook. Jap. Jour. Bot. **7**: 19. 1933.
5. POELLNITZ, KARL VON, Monographie der Gattung *Talinum* Adans. Repert. Spec. Nov. Fedde. **35**: 1–34. 1934.
6. ———, Versuch zur einer Monographie der Gattung *Portulaca* L. Repert. Spec. Nov. Fedde. **37**: 240–320. 1934.
7. SUGIURA, T., A list of chromosome numbers in angiospermous plants. II. Proc. Imp. Acad. Tokyo **12**: 144. 1936.
8. ———, Studies on chromosomes in higher plants with special reference to cytokinesis. I. Cytologia **7**: 544–595. 1936.
9. ———, Studies on chromosomes in higher plants with special reference to cytokinesis. II. Cytologia Fujii Jub. Vol. **2**: 845–849. 1937.
10. TISCHLER, G., Pflanzliche Chromosomen-Zahlen. Tabulae Biologicae **12**: 57–115. 1937.
11. TJEBBES, K., The chromosome numbers of some flowering plants. Hereditas **10**: 328–332. 1928.
12. WARMKE, H. E., A section-smear method for plant cytology. Stain. Technol. **16**: 9–12. 1941.

EFFECTS OF HIGH SALT CONCENTRATIONS ON GROWTH OF BEAN PLANTS

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Introduction

An earlier experiment (11) at this laboratory showed that beans are one of the most sensitive plants to a high concentration of salt. There was little quantitative difference in growth responses to added chloride and to sulphate salts if these were compared on an isosmotic basis. There was a definite tendency, however, toward premature senescence of the older leaves on the plants grown on a substrate containing a high concentration of chloride salts, a condition not shown in cultures with a high concentration of sulphate salts. The leaves of the latter seemed deeper green than those of the controls.

In this earlier experiment, specific responses to various cations were unobservable, since no one cation predominated in the added mixture of calcium, magnesium, and sodium salts. Although under natural conditions the salts in saline soils are almost always mixtures of several cations and anions, it is difficult to ascertain the specific effects of ions from a study of such mixtures. The present study, with single salts added to a basal nutrient solution, was initiated to determine the specific effects of relatively high concentrations of calcium, magnesium, sodium, chloride, and sulphate on plant growth. The bean plant was selected for this investigation.

METHODS.—Germinated Red Kidney bean seeds were placed on a paraffined gauze suspended over a tray of basal nutrient solution. Three days later the seedlings were transferred to water-culture equipment (2). The containers held 13 liters of solution, which was constantly aerated with carbon-tube aerators. The osmotic concentration was 0.5 atm. (tap-water salts included). The original pH was 5.7, maintained at pH 6.0 by the addition of small amounts of HCl to chloride series and H_2SO_4 to sul-

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phate series. The basal nutrient solution in each container had the following composition (me./l.):

Ca	Mg	Na	K	Cl	SO_4	H_2PO_4	NO_3
5.9	2.7	2.1	4.1	1.8	4.3	1.5	7.0

0.5 p.p.m. of B, Mn, and Fe, added as boric acid, manganese chloride, and ferric citrate, respectively.

Twenty-four hours after the seedlings were transplanted to the jars, those which were to receive added salts were given the initial salt increment—an amount sufficient to raise the osmotic concentration of the solution one atm. Cultures which were to have more than one increment were given sufficient to increase the osmotic concentration one atm. each day until the desired level was reached. Table 1 lists the salts studied and the number of milliequivalents per liter of each at the various osmotic concentrations. Owing to the very low solubility of $CaSo_4$, it was impossible to study this salt in the range of concentrations employed in this experiment.

TABLE 1

MILLIEQUIVALENTS OF SALT ADDED TO EACH LITER OF BASAL NUTRIENT SOLUTION TO GIVE TOTAL OSMOTIC CONCENTRATIONS OF 1.5, 2.5, 3.5, AND 4.5 ATM.

SALT	TOTAL ATM. OSMOTIC CONCENTRATION*			
	1.5	2.5	3.5	4.5
Na_2SO_4	39	78	117	156
NaCl	24	48	72	96
$CaCl_2$	32	64	96	128
$MgCl_2$	34	68	102	136
$MgSO_4$	70	144	225	312

* Osmotic concentration of basal nutrient solution, 0.5 atm.

In a series of concentrations of each salt added to basal nutrient solution, the relationship between osmotic concentration and specific electrical conductance was determined, so that frequent periodic determinations of concentration could be made by conductance measurement.

The experiment was started November 27, 1941, and the plants were harvested December 29, when the first flower buds were beginning to open. The plants were divided into (*a*) leaves, (*b*) stems (plus petioles and hypocotyl), and (*c*) roots. Green and dry weights were obtained.

The plants were grown in December, a time of year characterized by comparatively low light intensity. Admittedly, the quantitative and qualitative responses to salt would be different under high light and high temperature conditions. BROYER and HOAGLAND (1) have called attention to the important role of light and photosynthesis in determining the carbohydrate and salt status of the plant, which in turn conditions the salt uptake. In another current experiment attempting to use the bean plant as a "biological yardstick" for integrating all the climatic factors affecting growth, plantings are being made each week and the plants harvested 3 weeks from date of planting. The "standard" plants grown during the course of this experiment (December) weighed less than half as much as those grown in June of the following year.

Results

The appearance of the plants just prior to harvesting is shown in figure 1. The quality and quantity of growth produced in the presence of the magnesium salts differed radically from that of isosmotic concentrations of the other salts. There were, however, some specific responses to each salt.

Na_2SO_4 SERIES.—The quality and quantity of this plant growth was very similar to that in the NaCl and $CaCl_2$ series (fig. 1). The primary leaves were

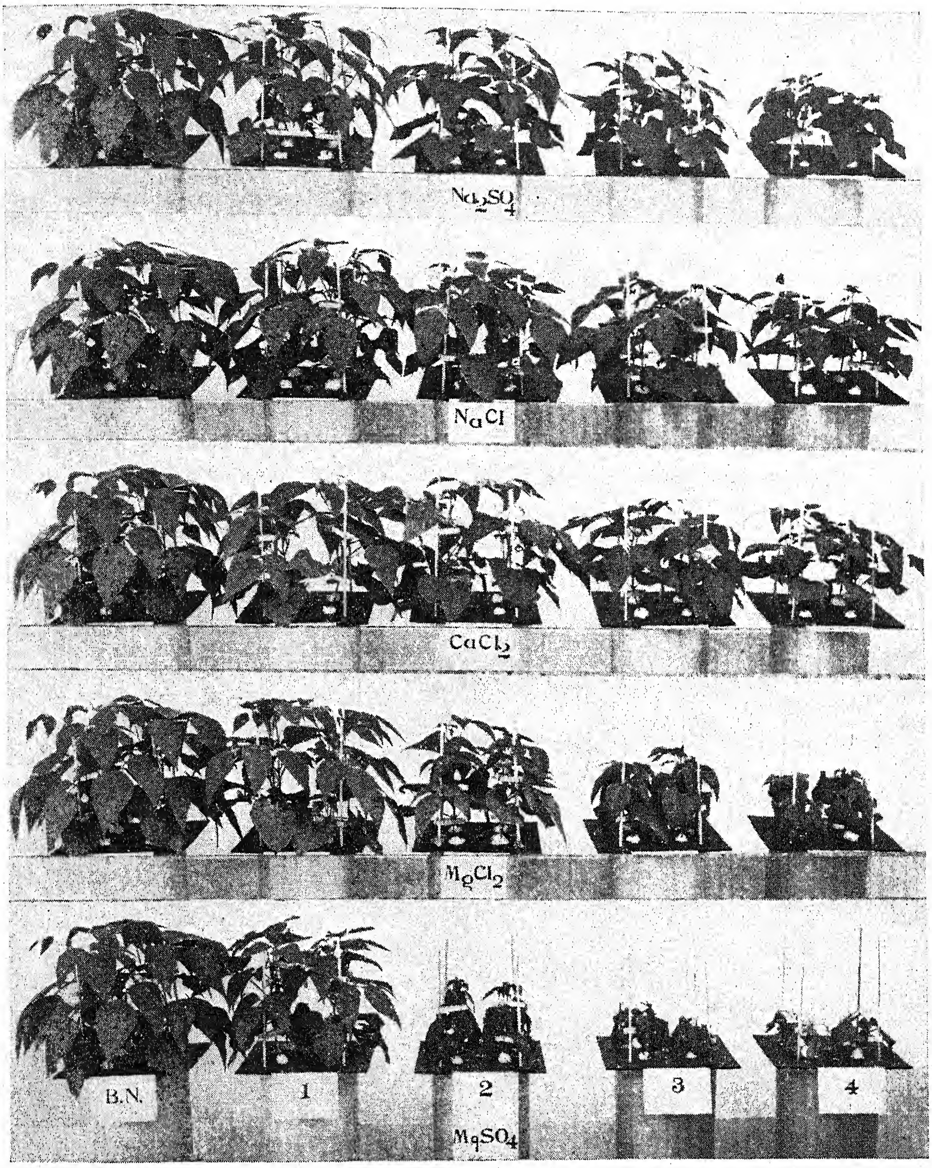

FIG. 1.—Appearance of plants at time of harvest. Figures on jars indicate atm. osmotic concentration of added salts; basal nutrient salts, 0.5 atm.

of the same color as those of the control plants, but the higher the concentration of Na_2SO_4 the more pronounced was the marginal and tip burn, resulting in brown necrotic tips and margins. The younger trifoliate leaves were darker green than those of the controls. The addition of the salt had no obvious effect on the type of growth or on the color of the roots.

NaCl SERIES.—At isosmotic concentrations, these plants resembled those of the Na_2SO_4 series in general appearance. In contrast with plants receiving Na_2SO_4, the primary leaves of these plants were yellow-green—lighter in color than those of the controls. As in the Na_2SO_4 series, the younger trifoliate leaves were darker green than those of the control plants. There were no observable specific effects of this salt on the roots.

$CaCl_2$ SERIES.—At isosmotic concentrations, these plants were similar in appearance to the Na_2SO_4 and NaCl series. The primary and first trifoliate leaves were yellow-green, while the second trifoliate leaf and the younger trifoliate leaves were yellow-green to green. In general, the plants were lighter in color than those of the controls of the Na_2SO_4 and NaCl series.

$MgCl_2$ SERIES.—In the presence of one atm. of added salt, the quantity and quality of growth was comparable with that of the three previous series. With twice this amount of salt, the symptoms were specific. The primary leaves and the first and second trifoliate leaves were very light in color (yellow-green), and the symptoms developed on these primary leaves were characteristic of those usually designated as "chloride burning." This burning differs from that which occurs with high concentrations of sulphate (for example, Na_2SO_4), in that it is not restricted to the tips and margins but frequently occurs in isolated patches on the primary leaves. Often there is some tip burn in addition to the isolated necrotic patches. Abortion of tertiary lateral roots was prevalent, and those tertiaries which did not early abort made a growth of only $\frac{1}{4}$–$\frac{1}{2}$ inch. When high magnesium concentration results in abortion of tertiary lateral roots, the loci of their primordia are represented by only brown dots.

At the 2-atm. concentration of $MgCl_2$ there was evidence of magnesium toxicity on the above-ground portion of only one out of twelve plants. In this one plant there was a slight necrosis (browning) in the pulvini directly below the base of the leaflets of the second trifoliate leaf.

At the next highest concentration (3 atm.) the magnesium toxicity symptoms were more pronounced. There was moderate to severe necrosis of all pulvini directly below the base of the blades. The primary leaves were curled under slightly at the tips, and the leaf blades seemed thicker and were leathery to the touch. The interveinal tissue was elevated. The roots were tan in color, and there was practically no development of tertiary roots. Soon after the terminal growing points of the primary and secondary roots became inactive or were killed, the root primordia just above the main root tips for a distance of 10–15 mm. became active and grew 2–10 mm. before the tips of these laterals in turn became inactive or died. This resulted in a rosette of roots near the tip of many of the primary and secondary roots.

At the highest concentration (4 atm.) the symptoms were even more acute. There were three very chlorotic trifoliate leaves on a much-shortened stem axis. The roots were brown, with many rosettes, and there was complete inhibition of tertiary roots.

$MgSO_4$ SERIES.—In the presence of 1

atm. of added salt, plant weights were about 10% less than with the other four salts. Distinct magnesium toxicity symptoms were in evidence, even at this lowest concentration. There was some necrosis in the pulvini at the base of the primary leaf blades and at the base of all trifoliate leaflet blades. At this concentration of $MgSO_4$ the necrosis in the pulvini was of limited extent and affected only a portion of the cortex on the abaxial surface of the pulvini. On about one-fourth of the plants there was browning or necrosis of the smaller veins on the third trifoliate leaf. The color of the roots was light tan, as in the 1-atm. $MgCl_2$ plants, and there was nearly complete inhibition of tertiary roots.

With 2 atm., the dry weight of the plants was 40% that of the 1-atm. plants and about 29% that of the controls. At the 1-atm. level the terminal leaflets of all trifoliate leaves measured more than 2 inches in breadth; but with twice as much salt, none of the terminal leaflets of any of the four trifoliate leaves measured 2 inches. The stem axis was much shortened (fig. 1). Necrosis in the pulvini regions was severe and in many of the primary leaves extended from the pulvinus at the base of the blade downward on to the petiole and centrifugally to the main veins of the primary leaves. There was a slight wilted appearance to the primary leaves. The roots were tan to brown, and there were practically no tertiary roots. There were a few rosettes of roots on some of the primary and secondary roots near the tips.

At the second-highest concentration (3 atm.) the dry weight of the plants was about 23% of that of the 1-atm. and about 16% of that of the control plants. In most cases there were only two trifoliate leaves, and the terminal leaflets of these did not attain 1 inch in breadth. All previously described symptoms were much more acute, including the "wilting" of the primary leaves.

With 4 atm., only one plant out of twelve was alive at the time the experiment was concluded.

Although the preceding descriptions have dealt with the appearance of the plants on the day preceding harvesting, the magnesium toxicity symptoms on the tops—and especially on the roots—were in evidence within a day after all solutions had been brought to full concentration. Naturally these symptoms became more pronounced with time.

Growth data

Average dry weights and percentage dry matter are given in table 2. At isosmotic concentrations, very similar amounts of top and root growth occurred in the presence of NaCl, $CaCl_2$, and Na_2SO_4 (fig. 2). In view of the different cations and anions involved in these three salts, the amount of growth was obviously not related to cation or anion specificity; rather the data show a linear relationship between growth and total osmotic concentration of the solution. Other work (3, 5, 6, 7, 8, 9, 11) has also indicated that for most salts or mixtures of salts the amount of growth is closely related to the osmotic concentration of the solution in contact with the roots.

The various salts diversely affected the percentage dry matter in the tops and roots (fig. 3). There was also a segregation of effects, that is, in the tops there was a grouping of the curves of the chloride salts *vs.* the sulphate salts. Thus in the tops the degree of "succulence" was apparently determined by the type of anion (Cl *vs.* SO_4). In the roots there tended to be a definite grouping, but in

TABLE 2

INFLUENCE OF SINGLE SALTS (ADDED TO BASAL NUTRIENT SOLUTION) ON DRY WEIGHT AND PERCENTAGE DRY MATTER OF BEAN PLANTS

Salt added	Total osmotic concentration (atm.)	Dry weight per plant (gm.)				Dry matter (%)		
		Leaves	Stems	Roots	Whole plant	Leaves	Stems	Roots
None	0.5*.......	3.71	1.57	1.21	6.49	10.94	8.85	5.45
Na_2SO_4	1.5........	3.20	1.38	1.09	5.67	11.46	9.71	5.29
	2.5........	2.50	1.08	0.92	4.50	12.00	10.25	5.30
	3.5........	1.77	0.75	0.69	3.21	12.51	10.86	5.68
	4.5........	1.21	0.55	0.54	2.30	13.29	11.64	6.20
NaCl	1.5........	3.24	1.39	1.13	5.76	10.62	9.34	5.32
	2.5........	2.62	1.20	0.97	4.79	10.88	9.87	5.24
	3.5........	2.11	0.91	0.74	3.76	10.12	10.44	5.20
	4.5........	1.52	0.68	0.58	2.78	10.96	11.05	5.32
$CaCl_2$	1.5........	3.13	1.35	1.00	5.48	10.96	9.93	6.16
	2.5........	2.70	1.16	0.93	4.79	10.36	10.43	6.66
	3.5........	1.85	0.81	0.60	3.26	10.95	11.04	6.62
	4.5........	1.58	0.66	0.54	2.78	11.29	11.62	6.71
$MgCl_2$	1.5........	3.13	1.40	0.98	5.51	10.14	9.07	6.27
	2.5........	2.17	0.86	0.55	3.58	10.85	10.13	7.04
	3.5........	1.19	0.47	0.31	1.97	10.66	10.89	8.08
	4.5........	0.79	0.23	0.18	1.20	(14.09)	12.39	7.61
$MgSO_4$	1.5........	2.79	1.15	0.75	4.69	12.21	9.99	7.67
	2.5........	1.13	0.43	0.32	1.88	16.38	13.79	9.50
	3.5........	0.67	0.23	0.16	1.06	17.67	15.53	8.73
	4.5........	92% mortality; no plants harvested						

* Basal nutrient.

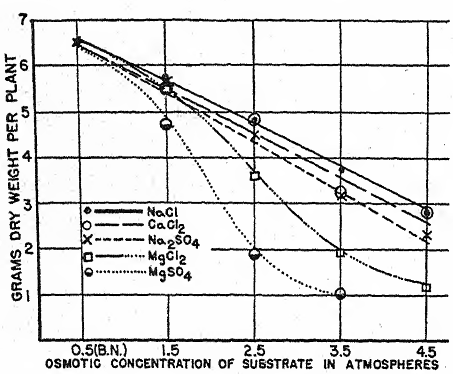

FIG. 2.—Average dry weight of bean plants as influenced by salts added to basal nutrient solution.

this case the comparison is monovalent sodium *vs.* the divalent cations, calcium and magnesium. In a previous paper (5) it was shown that when bean plants were subjected to high concentrations of NaCl, sodium predominated over calcium (on a milliequivalent basis) only in the roots—the only part of the plant in which there was a marked accumulation of sodium. The low percentage dry matter in the roots (high "succulence") associated with sodium and the high percentage dry matter associated with calcium or magnesium agree with the well-known effects of sodium *vs.* calcium and magnesium on the degree of hydration of colloidal material. As for the effect of chloride *vs.* sulphate on the percentage dry matter in the tops, HAYWARD and LONG (9) reported increased succulence in tomatoes associated with chloride and decreased succulence (higher percentage dry mat-

ter) associated with sulphate salts. The results of this experiment are in agreement with their findings.

Discussion

In the light of advances in technique within the past decade or two, most of the early work on the effect of various singly applied salts on the growth of plants was characterized by certain inadequacies. Usually only very small volumes of solution were available to the plant, and often the solution was unchanged and unaerated. The work of Broyer and Hoagland (1) and others has recently shown the necessity of adequate aeration for the process of salt absorption by plants. In many cases the individual salts have been added to distilled water only, and in the absence of all the macroelements and microelements it is difficult to apply or evaluate the data thus obtained. Those who added the salt to distilled water only were of necessity forced to conduct short-term experiments, and there are few data on plants carried to the flowering stage or to maturity. In some cases the investigator replaced one or more of the major salts of the basal nutrient with an added salt, thus using a solution deficient in one or more of the macroelements. Inasmuch as the amount of salt added varied, the degree of substitution of added salt for regular constituents of the basal nutrient solution was simultaneously a variable.

In the present study, the individual salts were added to a basal nutrient solution known to be adequate for at least a near-optimal growth of the bean plant. There were 13 liters of solution per container (supporting four plants), and the solution was continuously well aerated. Determinations were made of the osmotic concentration of the solutions, and the concentrations were maintained as nearly constant as possible.

Inasmuch as the necessary nutrient elements were supplied to all cultures in this experiment, any variations in the growth responses were believed to be strictly attributable to the individually added salts. Since certain specific-ion

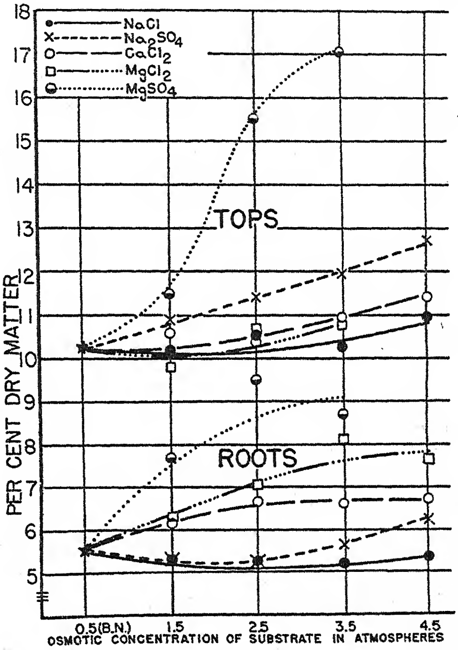

Fig. 3.—The influence of single salts (added to basal nutrient solution) on percentage dry matter in tops and roots of bean plants.

plant symptoms were obtained with the individual salts, these specific symptoms might be of diagnostic value in appraising the quality and something of the quantity of salt in the soils. It is not an uncommon situation to find in an irrigated soil a predominant accumulation of one salt, such as sodium chloride or sodium sulphate. In a study of seventeen representative western soils, Magistad and Reitemeier (10) found several soils having solutions in which the magnesium concentration exceeded that of

calcium. Although very low in total salt content, one of the more important agricultural soils in California contains more magnesium than calcium. The usual case, of course, is that when saline accumulations occur there is a mixture of accumulated salts. In a previous paper (13) it was pointed out that sodium chloride has an effect on nitrogen metabolism which is distinguishable from that produced by calcium chloride. It was possible to correlate the observed effects on nitrogen metabolism with (*a*) the uptake and concentration of sodium *vs.* calcium in the various parts of the bean plant and (*b*) the known effects of these two cations on the hydration of colloids. The factors determining salt uptake are not yet fully elucidated, however, and certainly the process is a complex one, as has been pointed out by BROYER and HOAGLAND (1).

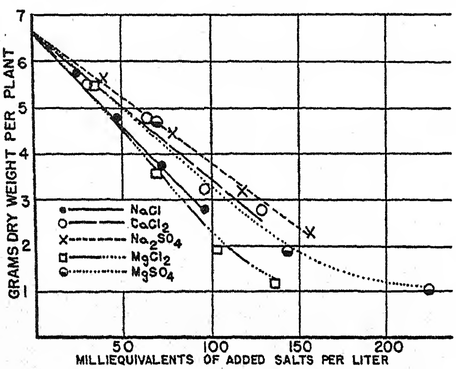

FIG. 4.—Average dry weight of bean plants as influenced by salts added to basal nutrient solution.

The marked difference in the effect of Mg upon the growth response as compared with that of Na or Ca is the striking feature of this study. It is evident in table 2 and in figure 3 that growth in the presence of a magnesium salt is markedly reduced as compared with growth in the presence of $NaCl_2$, $CaCl_2$, or Na_2SO_4. Inasmuch as this disparity appears with either $MgCl_2$ or $MgSO_4$, it is clearly a matter of specific cationic (magnesium) toxicity. Magnesium toxicity has been previously reported for wheat (12) and for Red Kidney bean (4).

In the present study, at isosmotic concentrations there was greater growth reduction in the presence of $MgSO_4$ than with $MgCl_2$. In terms of milliequivalents per liter, there was slightly more than twice as high a concentration of magnesium in the $MgSO_4$ solutions as in the $MgCl_2$ solutions. With respect to the specific action of magnesium, it is pertinent to consider the disparity in the amount of growth made in the presence of $CaCl_2$ *vs.* $MgCl_2$ (fig. 2) where essentially equal concentrations in equivalents of ions were involved (table 1). Thus it is evident that there is cationic specificity in the case of magnesium which operates in addition to the osmotic concentration effect of the solution. There is a rather close relationship between the concentration of magnesium (me./l.) and growth depression, but it must be assumed that other factors (for example, osmotic concentration, effect of associated anion on rate of uptake of magnesium, etc.) are also operative.

When growth of the plants in the magnesium salts is plotted against milliequivalents of salt per liter (fig. 4), $MgCl_2$ results in greater reduction in growth than does $MgSO_4$. Inorganic analyses of all samples have not been completed as yet, but a few were made, and these show that at iso-equivalent concentrations of $MgCl_2$ and $MgSO_4$, appreciably more magnesium was taken up when in association with chloride than when with sulphate. Higher concentrations of chloride were found in the leaves of $MgCl_2$ plants than in either the $CaCl_2$ or NaCl plants, which finding

would seem to be correlated with the pronounced "chloride burning" symptoms reported earlier in this paper for $MgCl_2$ plants. Over and above the effect of osmotic concentration on growth, high concentrations of magnesium thus produce specific symptoms on the tops and roots of bean plants.

It should be stressed that the specific symptoms noted in this paper are for the bean; other plants might not exhibit similar symptoms. The use of the bean plant as an indicator in studies of saline soils is suggested.

Summary

1. Red Kidney bean plants were grown to the flowering stage in aerated solution culture with basal nutrient solution and in this solution with various amounts of individual salts added.

2. The added salts were Na_2SO_4, NaCl, $CaCl_2$, $MgCl_2$, and $MgSO_4$. The effect of these separate salts on plant growth was studied in concentrations of 1.5, 2.5, 3.5, and 4.5 atm. osmotic concentration, including the 0.5 atm. concentration of basal nutrient.

3. At isosmotic concentrations very similar amounts of plant growth occurred in the NaCl, $CaCl_2$, and Na_2SO_4 series, but there was marked depression of growth with $MgCl_2$ and $MgSO_4$.

4. For most salts and mixtures of salts there tends to be a linear relationship between growth and osmotic concentration, expressed in atmospheres. The greatly depressed growth of plants in the presence of $MgCl_2$ or $MgSO_4$, as compared with that in NaCl, $CaCl_2$, and Na_2SO_4, is attributed to the specific toxicity of magnesium. Magnesium toxicity symptoms, as they occurred on the bean plant, are described.

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LITERATURE CITED

1. Broyer, T. C., and Hoagland, D. R., Metabolic activities of roots and their bearing on the relation of upward movement of salts and water in plants. Amer. Jour. Bot. 30:261–273. 1943.
2. Eaton, F. M., Plant culture equipment. Plant Physiol. 16:385–392. 1941.
3. ———, Water uptake and root growth as influenced by inequalities in the concentration of the substrate. Plant Physiol. 16:545–564. 1941.
4. Gauch, H. G., Response of the bean plant to calcium deficiency. Plant Physiol. 15:1–21. 1940.
5. Gauch, H. G., and Wadleigh, C. H., The influence of saline substrates upon the absorption of nutrients by bean plants. Proc. Amer. Soc. Hort. Sci. 41:365–369. 1942.
6. Gauch, H. G., and Magistad, O. C., Growth of strawberry clover varieties and of alfalfa and Ladino clover as affected by salt. Amer. Soc. Agron. 35:871–880. 1943.
7. Hayward, H. E., and Long, E. M., Anatomical and physiological responses of the tomato to varying concentrations of sodium chloride, sodium sulphate, and nutrient solutions. Bot. Gaz. 102:437–462. 1941.
8. ———, and ———, Vegetative responses of the Elberta peach on Lovell and Shalil rootstocks to high chloride and sulfate solutions. Proc. Amer. Soc. Hort. Sci. 41:149–155. 1942.
9. ———, and ———, Some effects of sodium salts on the growth of the tomato. Plant Physiol. 18:556–569. 1943.
10. Magistad, O. C., and Reitemeier, R. F., Soil solution concentrations at the wilting point and correlation with plant growth. Soil Sci. 55:351–360. 1943.
11. Magistad, O. C., Ayers, A. D., Wadleigh, C. H., and Gauch, H. G., The effect of salt concentration, kind of salt, and climate on plant growth in sand cultures. Plant Physiol. 18:151–166. 1943.
12. Trelease, S. F., and Trelease, Helen M., Magnesium injury of wheat. Bull. Torr. Bot. Club 58:127–148. 1931.
13. Wadleigh, C. H., and Gauch, H. G., Assimilation in bean plants of nitrogen from saline solutions. Proc. Amer. Soc. Hort. Sci. 41:360–364. 1941.

MYCORHIZAS OF FILBERT AND WALNUT TREES IN OREGON ORCHARDS[1]

C. E. SCHUSTER,[2] R. E. STEPHENSON,[3] AND WILLIAM EVENDEN[4]

Introduction

Walnut and filbert trees in orchards of Oregon show the development of mycorhizas in sufficient abundance to justify a survey to determine the types involved and their prevalence. In 1934, studies of roots of filbert and walnut trees in orchards of the Willamette Valley in Oregon were begun by SCHUSTER and STEPHENSON. The investigation included determination of the distribution of the roots within each soil horizon. In some soils roots were restricted to very limited depths; and apparently typical root absorptive regions (root-hair zones) were inadequate for satisfactory plant development, as the quantity of roots recovered from the soil was very small compared with that found for some other fruit trees.

FRANK (2) proposed as early as 1885, and also subsequently, that both endotrophic and ectotrophic forms of mycorhizas were capable of modifying root absorptive regions to the advantage of the host plants. Later, HATCH (4) and MITCHELL, FINN, and ROSENDAHL (7) showed that ectotrophic mycorhizas are capable of aiding in the nutrition of specific forest trees in definite soil types. The influence of an endophytic fungus on the germination of orchid seed has been shown (5) to be related to the nutrition of the developing embryo. Other cases of assumed benefit or detriment from ectotrophic or endotrophic mycorhizas have largely remained unproved.

METHODS.—In determining the prevalence of mycorhizas, several hundred root samples were taken at random from nine filbert and twelve walnut orchards, representative of most of the soil types on which these trees are grown in the Willamette Valley. Since, according to SCHUSTER and STEPHENSON (10), the greatest concentration of roots occurs generally in the first or second foot, samples were collected for the most part from the upper 2 feet of soil, with sufficient material from lower depths to establish the presence or absence of mycorhizas.

The roots were washed free of loose soil in a large-mesh sieve and stored in water for several hours to loosen adhering soil particles. They were then inclosed by fine-mesh wire screen and washed thoroughly with a strong stream of tap water. The desired parts were removed from the washed material with forceps and scissors and kept submerged in water or in killing and fixing solution at all times.

In preparation for histological study, the material was fixed in weak chromacetic fixative for 48 hours (1) or for 24 hours (9), then dehydrated in dioxan and imbedded in paraffin in the usual way and cut 8–10 μ thick. Preparation for staining was according to GALIGHER (3) and the staining was in Delafield's haematoxylin.

Results

ECTOTROPHIC MYCORHIZAS OF CORYLUS AVELLANA L.

Ectotrophic mycorhizas are so generally present on filbert roots (fig. 1) that

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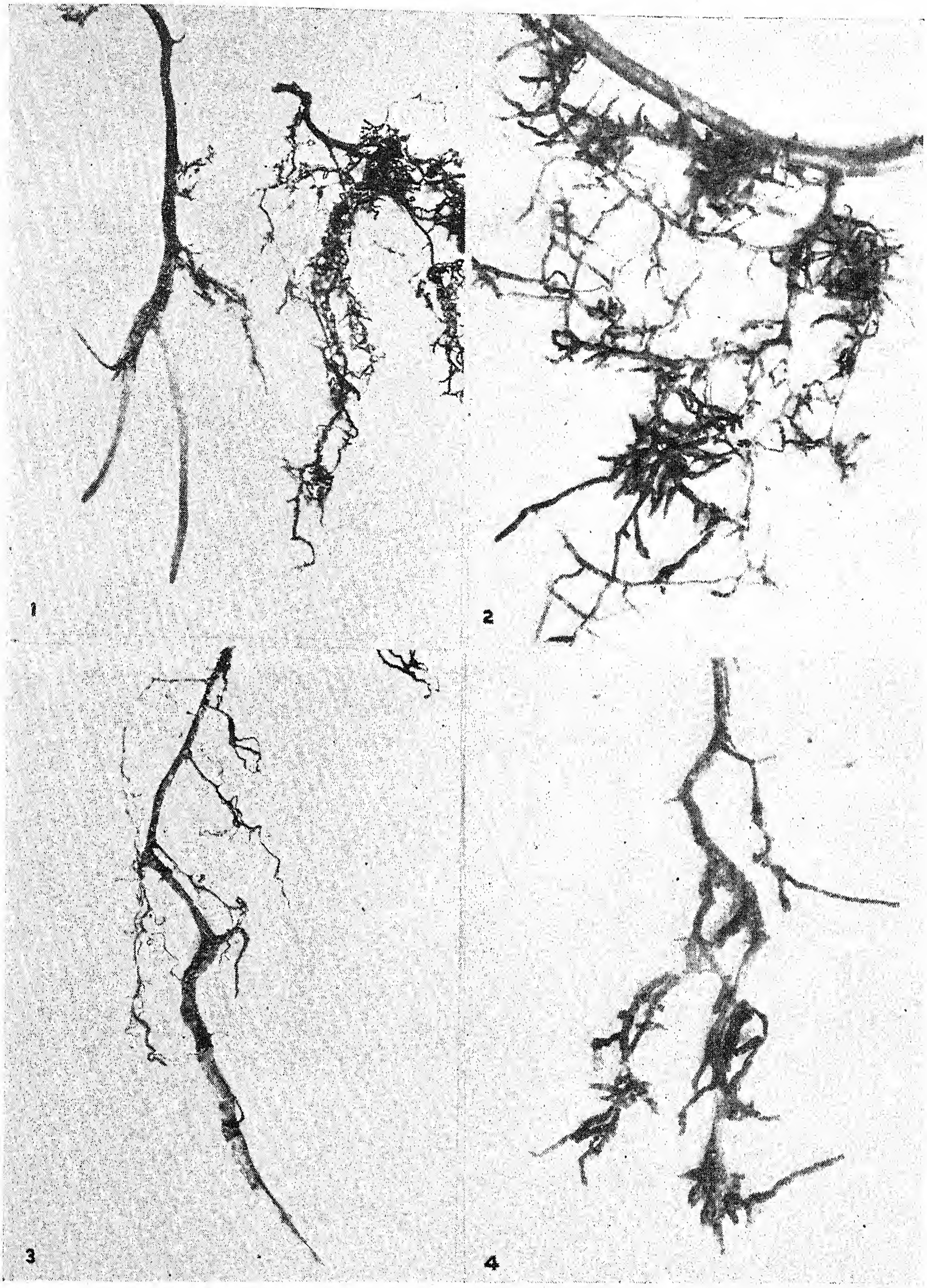

FIGS. 1–4.—Fig. 1, mycorhizal and nonmycorhizal roots of filbert; root at left has two long nonmycorhizal tips; remainder are ectotrophic mycorhizas. Fig. 2, ectotrophic mycorhizal roots of filbert; note excessive branching. Fig. 3, mycorhizal and nonmycorhizal roots of walnut. Long terminal root is nonmycorhizal; others are old endotrophic mycorhizas. Fig. 4, endotrophic mycorhizal roots of walnut; note clubbing and distortion.

their effects on root development cannot be determined under orchard conditions. Studies were made, however, of the differences in structure between mycohizal and noninfected roots. Ectotrophic mycorhizas have not been found in the case of walnut.

Mycorhizal condition of roots is indicated by cessation of terminal growth and clubbing of the laterals (fig. 2). In turn, these laterals make only limited terminal growth, a condition which seems to encourage formation of another series of short laterals. The final result is a dense, much-branched system of short roots, whereas noninfected roots are long and only slightly branched. The surface of each root is covered by a mantle of fungus growth. Within this is another layer of hyphae, the Hartig net, formed by the hyphae penetrating intercellular spaces of the epidermal layer to a depth of one or two cells. From this layer develops the mantle just mentioned. The root tissues below the Hartig net are noninfected.

Endotrophic mycorhizas of Juglans regia L.

While these data are from Persian walnut orchards, the roots examined are from seedlings of the northern California black walnut (*Juglans hindsii* Sarg.) used as rootstock. There are no data regarding roots of Persian walnut seedling trees. Endotrophic mycorhizas have not been found in the case of filbert.

The effect of the endotrophic mycorhiza on the growth of walnut roots is similar to that produced in those of filbert affected by ectotrophic fungi, in that terminal growths are shortened and laterals clubbed and shortened (figs. 3, 4). A larger proportion of noninfected roots is found than in the case of filbert, but the fungi are never absent from more than a few terminal growths, and apparently this noninfected condition is only temporary. In young endotrophic mycorhizas of walnut there is no outer layer of fungus but instead epidermal cells devoid of root hairs and without cork. Within the epidermis is the infected region, where the hyphae are intracellular —in contrast to the condition found in filbert, where the ectotrophic hyphae are intercellular.

The endotrophic fungus or fungi have sterile nonseptate mycelia of phycomycetous form, with at least two sizes of hyphae.

Occurrence of mycorhizas

Mycorhizas on walnut and filbert roots were collected from orchards growing on Aiken, Olympic, Melbourne, Salkum, and Willamette clay loam; on Aiken, Sites, Willamette, Newberg, and Amity silty clay loam; on Newberg and Chehalis loam; and on Newberg fine sandy loam soil. This list includes all the series of better soils for nut production and some mediocre soils on which nut orchards are occasionally found in Oregon. Mycorhizas were always present on walnut or filbert roots, regardless of the soil type or of the amount of available phosphorus, soluble potassium, soluble calcium, organic matter, or pH of the soil.

During the first year of study by the senior writers, no unaffected growing tips of either walnut or filbert roots were found. In the succeeding year, however, a few white, apparently noninfected roots on walnuts were present at all times of the year after early summer. In that investigation no histological studies were made.

No attempt was made to identify the

fungi; the study was concerned only with their presence and distribution. Presence of the fungus or fungi may be explained in two ways. Filbert trees imported from Europe have been the foundation stock for most orchards in the Pacific Northwest. FRANK (2), LECOMTE (6), and PEYRONEL (8) record mycorhizas on *Corylus avellana* L. of Europe, and these may have been introduced on such imported stock. Also, since young trees are generally propagated by layerage from bearing trees or from stools especially used for the purpose, it is probable that further dissemination occurred. However, mycorhizas have been found on the native hazel, *Corylus californica* (Rose), indicating that the same or related species of fungi are indigenous. In the case of the walnut, the fungus or fungi are probably indigenous, since practically all the rootstock material consisted of seedlings produced in the Willamette Valley, grown from nuts locally produced or from California.

Discussion

On shallow infertile soils, trees are small, low in production, and have a greater rate of mortality than trees on the deep fertile soils. In these shallow soils the roots are confined to the upper levels, and roots of walnut or filbert trees growing thereon are almost completely infected with mycorhizas. In deep fertile soils the total quantity of roots per tree is much greater than in shallow soils, but in the upper levels of both types of soil the quantity of roots is approximately equal. In deep soils the mycorhizas are more widely and deeply distributed than in shallow soils.

Regardless of soil type, degree of fertility, or physical or chemical condition of the soil, mycorhizas are equally abundant in all soils on which walnut and filbert trees are growing. Under field conditions there is no evidence that in the deep fertile soils mycorhizas are either necessary or detrimental to the development of large productive trees, or that mycorhizal development on weak devitalized trees growing on shallow infertile soil has hastened or otherwise promoted their decline. Since some investigators claim the function of root hairs for both ectotrophic and endotrophic mycorhizal hyphae, it is assumed that this role is played in the case of walnut and filbert, regardless of type, depth, or physical or chemical quality of the soil.

The writers are indebted in various ways to A. B. HATCH, S. M. ZELLER, W. M. ATWOOD, C. E. OWENS, and J. B. SPULNIK; and for the excellent preparations from which the histological findings were made to MRS. E. R. GEIL.

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LITERATURE CITED

1. CHAMBERLAIN, C. J., Methods in plant histology. 4th ed. Univ. of Chicago Press. 1924.
2. FRANK A. B., Ueber die physiologische Bedeutung der Mycorhiza. Ber. deutsch. Bot. Ges. 6:248–268. 1888.
3. GALIGHER, A. E., Essential of practical microtechnique. Laboratory of microtechnique. Albert E. Galigher, Berkeley, California. 1934.
4. HATCH, A. B., The physical basis of mycotrophy in *Pinus*. Black Rock Forest Bull. 6. 1937.
5. KNUDSON, L., Non-symbiotic germination of orchid seeds. BOT. GAZ. 73:1–25. 1922.
6. LACOMTE, H., Note sur le mycorhize. Bull. Soc. Bot. France 34:38–39. 1887.
7. MITCHELL, H. L., FINN, R. F., and ROSENDAHL, R. O., The relation between mycorhizas and the growth and nutrient absorption of coniferous seedlings in nursery beds. Black Rock Forest Papers 1:10. 1937.

8. Peyronel, B., Nouveaux cas de rapports mycorhiziques entre phanérogames et Basidiomycètes. Bull. Soc. Mycol. France 37:143–146. 1921.

9. Schuster, C. E., Methods for softening filbert buds imbedded in paraffin. Bot. Gaz. 102:815–818. 1941.

10. Schuster, C. E., and Stephenson, R. E., Soil moisture, root distribution, and aeration as factors in nut production in western Oregon. Ore. Agr. Exp. Sta. Bull. 372. 1940.

BORON DEFICIENCY AND THE ASCORBIC-ACID CONTENT OF TOMATOES

C. B. LYON AND R. Q. PARKS

A series of investigations concerned primarily with the effects of mineral nutrition on the ascorbic-acid content of tomatoes have been completed at this laboratory. Large variations in the supply of calcium, potassium, magnesium, nitrate, sulphate, and phosphate produced relatively little effect on the ascorbic-acid content of tomato fruits (3). Similarly, lack of copper, iron, manganese, zinc, and molybdenum in the substrate resulted in no change of any practical importance in the vitamin content of fruits (4). In the latter study, specialized Pyrex apparatus was used which precluded a study of boron deficiency. It is the purpose of this note to record the ascorbic-acid content of tomatoes from boron-deficient plants grown in sand culture. The effects of a toxic supply of the micronutrients on the vitamin content of tomatoes will be reported elsewhere.

On May 1, 1943, seed of an inbred strain of Bonny Best tomatoes was germinated in the greenhouse in small crocks filled with pure quartz sand. The seedlings were supplied with nutrient solution of the following composition (p.p.m.): Ca, 285; K, 223; Mg, 69; N, 168; S, 144; P, 70; Fe, 5.0; Mn, 0.5; Mo 0.5; Zn, 0.05; Cu, 0.02. Eighteen seedlings were transplanted into 2-gallon glazed crocks with one seedling per crock on May 20, and all plants were placed outdoors. Nine plants were supplied with this nutrient solution throughout their life span; the remaining nine plants were supplied with the same nutrient containing in addition 0.5 p.p.m. of B. The first four fruits to mature on each plant were harvested on the morning of the day that complete color change had occurred and immediately analyzed for ascorbic acid by methods previously described (3). All plants were harvested August 21, when they were 112 days old.

The comparison of various characteristics for boron-deficient and control plants is given in table 1. Significantly less growth and fruitfulness occurred when boron was omitted from the nutrient medium. In addition, severe deficiency symptoms similar to those obtained in previous investigations were observed on vegetative parts of the plant (1, 2) and on fruits (2, 5). When all plants were harvested at the conclusion of the experiment, leaflet material of each plant was analyzed in duplicate for boron content. Leaflets from deficient plants averaged 7.6 ± 1.19 p.p.m. of boron on a dry-weight basis, while those from control plants contained 91.8 ± 3.30 p.p.m.

The average of twenty-six analyses of boron-deficient fruits was 25.9 ± 0.86 mg. of ascorbic acid per 100 gm. of fresh weight, while the average of thirty-six

analyses of the control fruits was 26.6 ± 0.58 mg. Within the limits of error there is no statistically significant difference in these means.

The lack of boron in this experiment resulted in less growth and fruitfulness, accompanied by severe deficiency symptoms with less boron in vegetative parts of the plant, but did not significantly affect ascorbic-acid content of fruits.

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TABLE 1

MEASUREMENTS OF GROWTH AND FRUITFULNESS GIVING TREATMENT MEANS TOGETHER WITH THEIR STANDARD ERRORS

PLANT CHARACTER	TREATMENT*	
	Boron deficiency	Control
I. Vegetative growth		
Height of vine (cm.)	67.7 ± 5.74	109.6 ± 4.39
Fresh weight of vine (gm.)	200.5 ± 23.10	356.0 ± 18.63
Dry weight of vine (gm.)	37.1 ± 4.60	68.7 ± 3.18
Percentage dry matter	18.2 ± 0.75	19.4 ± 0.50
Dry weight of roots (gm.)	4.7 ± 0.49	9.0 ± 0.70
II. Fruitfulness		
Total fresh weight ripe fruit per plant (gm.)	331.4 ± 106.71	831.7 ± 63.76
Number of fruit ripened per vine	4.9 ± 1.21	9.7 ± 0.81
Size ripe fruit (gm./fruit)	65.9 ± 14.77	88.5 ± 6.91
Number of immature fruit per vine at end of experiment	0.2 ± 0.22	9.3 ± 1.38
Total fresh weight of immature fruit per vine (gm.)	4.3 ± 4.30	499.1 ± 70.48
III. Analytical results		
Boron content of leaflets (γ/gm. dry wt.)	7.6 ± 1.19	91.8 ± 3.30
Ascorbic-acid content of fruit (mg./100 gm. fresh wt.)	25.9 ± 0.86 (26)	26.6 ± 0.58 (36)

* In any inter-treatment comparison (except for ascorbic acid), 16 degrees of freedom are available. When $t = 2.12$, $P = 0.05$; when $t = 2.92$, $P = 0.01$. In ascorbic-acid comparison, number of analyses for each mean is indicated in parentheses.

LITERATURE CITED

1. JOHNSTON, E. S., and DORE, W. H., The influence of boron on the chemical composition and growth of the tomato plant. Plant Physiol. 4:31–62. 1929.
2. JOHNSTON, E. S., and FISHER, P. L., The essential nature of boron to the growth and fruiting of the tomato. Plant Physiol. 5:385–392. 1930.
3. HAMNER, K. C., LYON, C. B., and HAMNER, C. L., Effect of mineral nutrition on the ascorbic-acid content of the tomato. BOT. GAZ. 103:586–615. 1942.
4. LYON, C. B., BEESON, K. C., and ELLIS, G. H., Effects of micronutrient deficiencies on growth and vitamin content of the tomato. BOT. GAZ. 104:495–514. 1943.
5. RALEIGH, G. J., Fruit abnormalities of tomatoes grown in various culture solutions. Proc. Amer. Soc. Hort. Sci. 37:895–900. 1939.

ANATOMICAL RESPONSES OF TOMATO STEMS TO VARIATIONS IN THE MACRONUTRIENT ANION SUPPLY

C. B. LYON AND C. R. GARCIA

Introduction

Numerous studies have been made on the anatomy of the tomato plant (4). Many of these studies, however, have been concerned primarily with descriptive or developmental anatomy, and the anatomy of the plant as it may be correlated with physiological responses has received comparatively little attention. KRAUS and KRAYBILL (6) correlated differences in the anatomy of tomato stems with nitrogen supply and the carbohydrate metabolism of the plant. HAYWARD and LONG (5) found differences in the cross-sectional area of tissue systems in tomato stems to be related to toxic concentrations of NaCl and Na_2SO_4 in the nutrient medium. LYON (7) reported hereditary variations in the extent of the tissue systems in tomato stems. The relative areas of these systems in three strains were not altered by additions of Na_2SO_4 in toxic quantities to the nutrient medium.

The present study was undertaken to examine anatomical differences in tomato stems which result from variations in the relative proportions of macronutrient ions supplied to the plant and to correlate observed differences with the vegetative and fruitful condition of the plant. The results with respect to variations in the nitrate, sulphate, and phosphate supplies are reported in this paper.

Material and methods

The material used was obtained from the 1941 experiment of HAMNER, LYON, and HAMNER, described in detail elsewhere (3). Briefly, an inbred strain of Bonny Best variety of tomatoes was planted in the greenhouse May 12, 1941, and the seedlings were transplanted into 2-gallon glazed crocks containing pure quartz sand. On July 1, all plants were placed outdoors. The plants were harvested September 10.

The design of the experiment was that of a randomized block (1) with eighty-seven treatments and four replications. Each replication consisted of a three-plant row, and the replications were randomized by the use of TIPPETT'S randomization tables (10). Thus, the mean of twelve plants is used as an estimation of the results produced by a given treatment. The data were reduced by means of the analysis of variance, and the t test (9) was used for determining whether particular differences were significant. Odds as great as or greater than 99:1 were accepted as statistically significant that the observed deviations were not due to the errors of random sampling. Subsequent to the time the seedlings were transplanted, the same randomization and design was maintained, both in the greenhouse and in the field.

In the forty-four solutions considered in this investigation, the relative proportions of nitrate, sulphate, and phosphate were varied, while the total equivalent concentration of these ions was constant. Each solution contained 12.0 milliequivalents per liter of calcium, 4.5 m.e./l. of potassium, and 9.0 m.e./l. of magnesium. For convenience, the forty-four solutions are represented in an anion triangle (fig. 1), and treatment numbers are assigned to those solutions used. All solutions contained equal amounts of the microelements in the following concentrations:

B (as HBO_3)	0.5	p.p.m.
Mn (as $MnCl_2 \cdot 4H_2O$)	0.5	
Zn (as $ZnSO_4 \cdot 7H_2O$)	0.05	
Cu (as $CuSO_4 \cdot 5H_2O$)	0.02	
Fe (as ferric citrate)	5.0	

When the plants were 120 days old and had received their respective nutrient treatments for 94 days they were harvested, and a section of the stem from the middle internode of each plant was preserved for an anatomical study. The stem sections were fixed with Navashin's solution, and air was evacuated from the tissues. The material was dehydrated in an ethyl-tertiary butyl-alcohol series and infiltrated with a paraffin-beeswax rubber mixture. Complete cross sections were

In these measurements the internal phloem was disregarded, and the term "pith" includes all cells internal to primary xylem; "xylem" includes all cells from primary xylem to cambium; "phloem" includes all cells from cambium to pericycle; "cortex" includes all cells from endodermis to and including cuticle.

The mean radial measurement of any tissue system for a given stem section was used to calculate the actual area of that tissue system in the cross section.

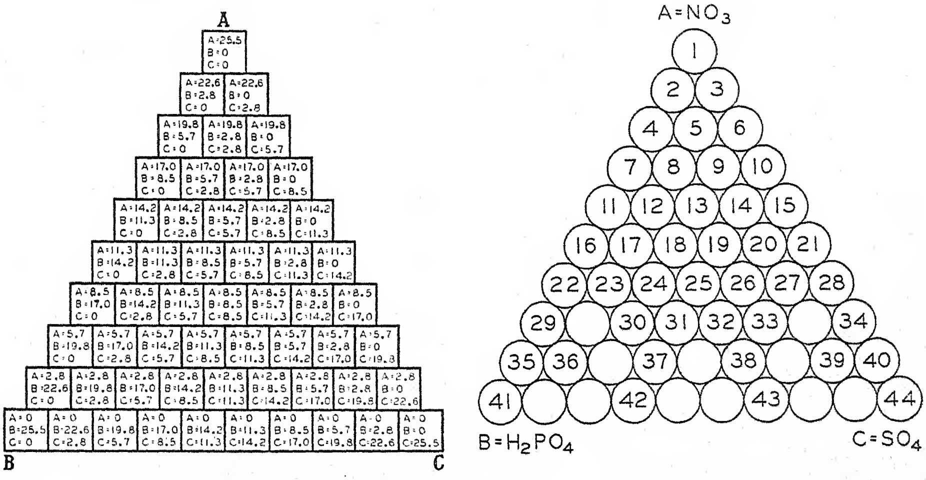

FIG. 1.—Left, anion composition in milliequivalents per liter of nutrient solution for 55 possible treatments. Right, treatments used, with treatment numbers assigned.

cut at 10–20 μ and stained with a modified Flemming's triple stain. The material was mounted in balsam.

Each stem section was projected so that the resulting image was enlarged approximately thirty diameters. The images were centered on a screen marked off with four diameters dividing the stem section into eight equal sectors (fig. 2). The radial measurements of each tissue system on each of the eight radii were recorded. Thus, the mean of eight measurements was used for xylem, phloem, and cortex; the mean of four measurements was used for pith and stem diameter.

Standard formulas developed for circles were employed. It was considered that the variability within a stem attributable to the deviations of the tissue systems from circles was nullified by the use of mean values. Thus, all inter-treatment comparisons consider only differences between stems with respect to areas of the tissue systems. Two types of inter-treatment comparisons were made for each tissue system: (*a*) actual areas were computed and treatment means compared, and (*b*) the percentage cross-sectional area of the stem section occupied by the area of any given tissue system was computed and treatment means compared.

Thus, the actual size as well as the relative amount of tissue in a stem section was evaluated.

Results

Photomicrographs of cross sections from typical tomato stems in each treatment are given in figure 3. Great differences—which include stem size, tissue system development, starch accumulation, and to some extent cellular anatomy—are demonstrable. These characteristics will be discussed individually.

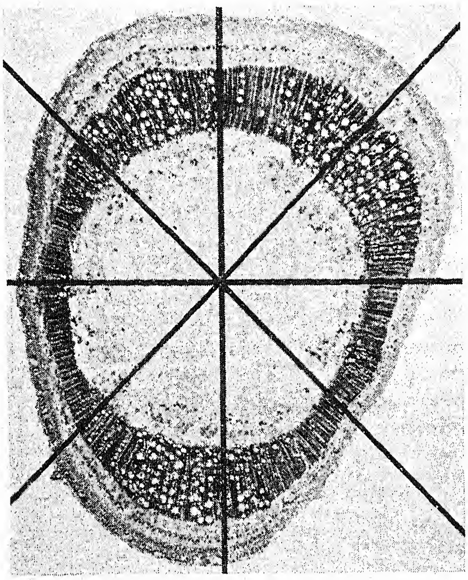

FIG. 2.—Radii used to obtain radial measurements of each tissue system in each stem section. Radii separated by 45° angles.

This experiment had been specifically designed so that data obtained for any character could be analyzed for the presence or absence of statistically significant differences. Accordingly, analyses of variance were computed for each individual character, and the results are compiled in table 1. Several plants were discarded as a result of mechanical injury resulting in a non-orthogonal experimental design. The sources of variation are:

VARIATION DUE TO	NO. OF DEGREES OF FREEDOM
Treatments	43
Between blocks	3
Within blocks	2
Error	427
Total	475

The substitution of missing values by YATES method (11) did not significantly change the results of analyses presented in table 1, and they are considered valid.

TABLE 1

F VALUES FROM ANALYSES OF VARIANCE FOR ANATOMICAL CHARACTERS OF TOMATO STEM CROSS SECTIONS

ANATOMICAL CHARACTERS	SOURCE OF VARIATION		
	Between treatments	Between blocks	Within blocks
Percentage			
Cortex	4.24*	1.36	0.69
Phloem	17.68*	2.75†	9.78*
Xylem	18.72*	1.28	7.97*
Pith	24.37*	1.95	12.72*
Area			
Cortex	28.39*	4.93*	4.16†
Phloem	39.12*	6.93*	8.69*
Xylem	44.43*	4.65*	4.49†
Pith	14.43*	2.17	0.75
Stem diameter	47.33*	3.92*	7.13*

* Highly significant, satisfying statistical requirements for $P = 0.01$.

† Possibly significant, satisfying statistical requirements for $P = 0.05$.

It is evident that treatments produced statistically significant differences in all the characters listed. It is also evident that differences in environment—when measured by replications both between and within blocks—produced significant differences in all characters except the percentage cross-sectional area occupied by the cortex and the actual area of the pith. Since replication differences were measurable in the analysis of variance,

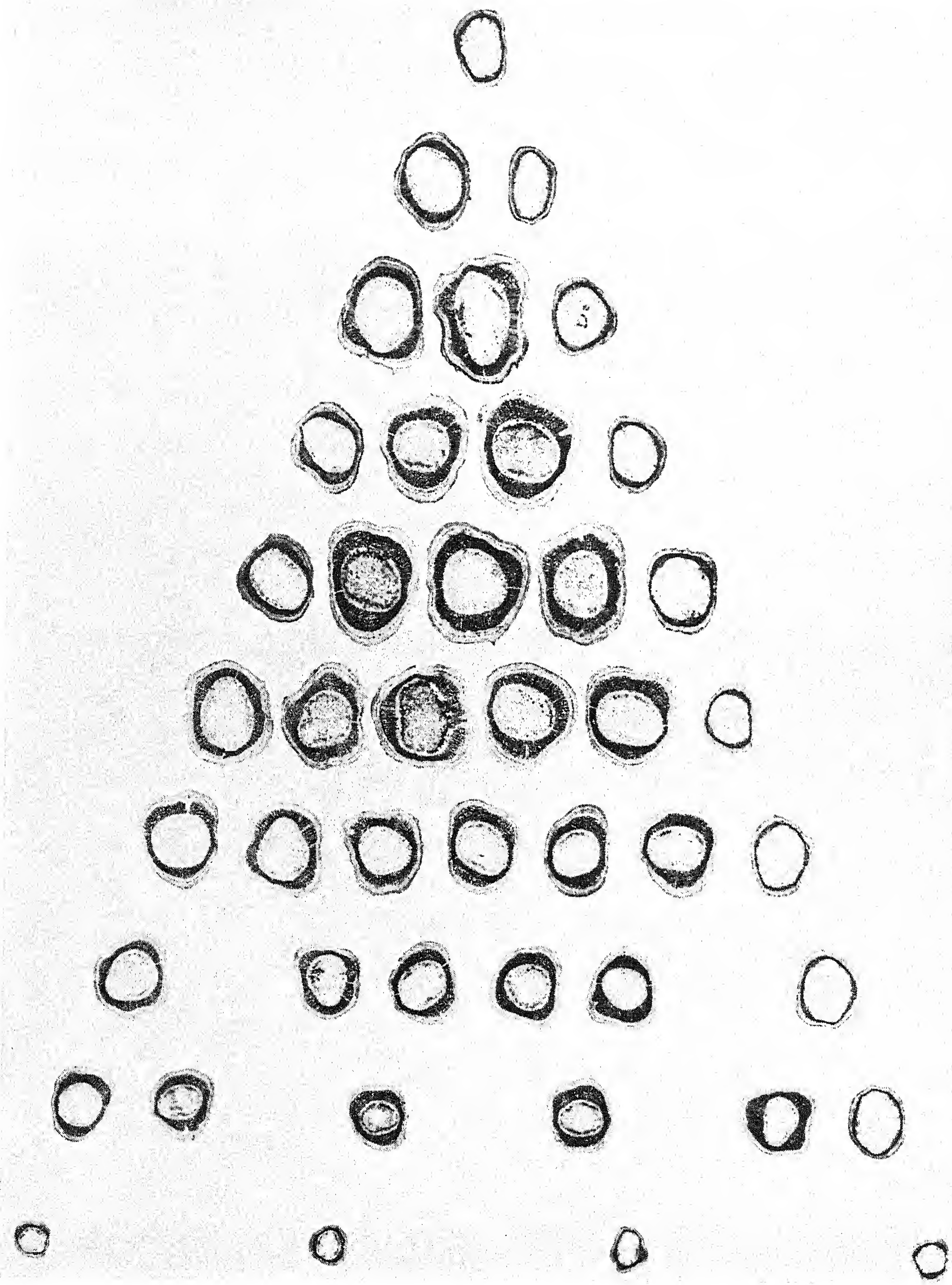

FIG. 3.—Typical cross sections of tomato stems from plants in anion treatments. Treatments correspond to those shown in fig. 1.

and since these differences were not included in inter-treatment comparisons, valid differences exist in all the listed characters which can be ascribed directly to the treatments.

An inter-treatment comparison of stem diameters is given in figure 4. Plants in treatments receiving no nitrate subsequent to the seedling stage had the smallest stems of any in the experiment.

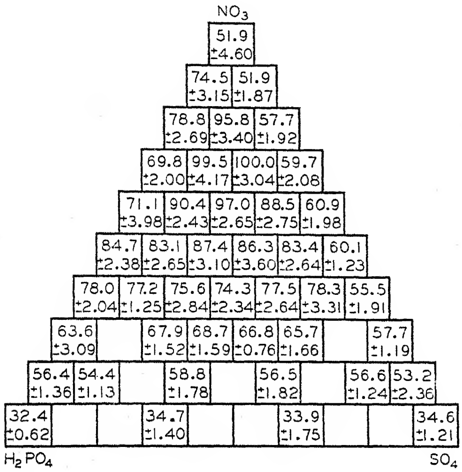

FIG. 4.—Inter-treatment comparison of stem diameters. Treatment no. 9, with actual stem diameter of 10.7 mm., designated 100 and all other treatments related to it. Treatment means, together with standard errors, given.

Significantly larger stems were noted with each successive increment of nitrate supply, and the largest were obtained in treatment no. 9, receiving 17.0 m.e./l. of nitrate in the nutrient solution. However, plants receiving nitrate as the only macronutrient anion (treatment no. 1) compared unfavorably with optimum treatments in the central portion of the triangle with respect to this character. Stem size was significantly less than optimum in all phosphate-deficient treatments and also in all sulphate-deficient treatments.

From figure 5 it is evident that the area of each of the component tissue systems varies in a manner similar to that observed for stem diameter. Without exception, nitrate-deficient plants have the smallest tissue systems of any, and the areas of each of the component tissue systems increase with increments of nitrate supply. Maximum values are obtained in those treatments receiving 17.0 m.e./l. of nitrate in the nutrient medium. Values for phosphate-deficient treatments are significantly less than those for optimum treatments shown in the central portion of the triangle. Little variation in phosphate-deficient treatments as a result of altered nitrate and sulphate supply is indicated. While values for all sulphate-deficient treatments are significantly less than maximum, significant variations occur as a result of differences in nitrate and phosphate supply.

TABLE 2

CORRELATION COEFFICIENTS BETWEEN INDICATED VARIABLES IN CROSS SECTIONS OF TOMATO STEMS

ANATOMICAL CHARACTERS	AREA			STEM DIAMETER
	Phloem	Xylem	Pith	
Area*				
Cortex	+0.977†	+0.950†	+0.493†	+0.966†
Phloem	....	+0.978†	+0.853†	+0.952†
Xylem	....	....	+0.542†	+0.952†
Pith	....	....	....	+0.934†

* Actual area of indicated tissue system in stem cross section.

† Highly significant, satisfying statistical requirements for P = 0.01.

The correlations involving areas of tissue systems and stem diameter as variables are given in table 2. It is evident that highly significant and positive correlations exist between each of the component tissue systems and stem diameter. The magnitude of the correlation coefficients is unusually high. Thus,

an increase in stem diameter and hence cross-sectional area of stems as a result of treatment is the result of a corresponding increase in the area of each of the component tissue systems. The areas of the tissue systems are also inter-correlated among themselves by highly significant coefficients. It is noticeable that two of the correlations involving pith as a variable are less striking in their magnitude.

While the actual amount of cortex was highly variable as a result of treatment, the percentage cross-sectional area of the stem section occupied by the area of the cortex remained relatively constant (fig. 6). Although the mean differences are statistically significant, as shown by the analysis of variance (table 1), the magnitude of the differences is small and very little correlation with nutrient supply is evident. There is some indication that a

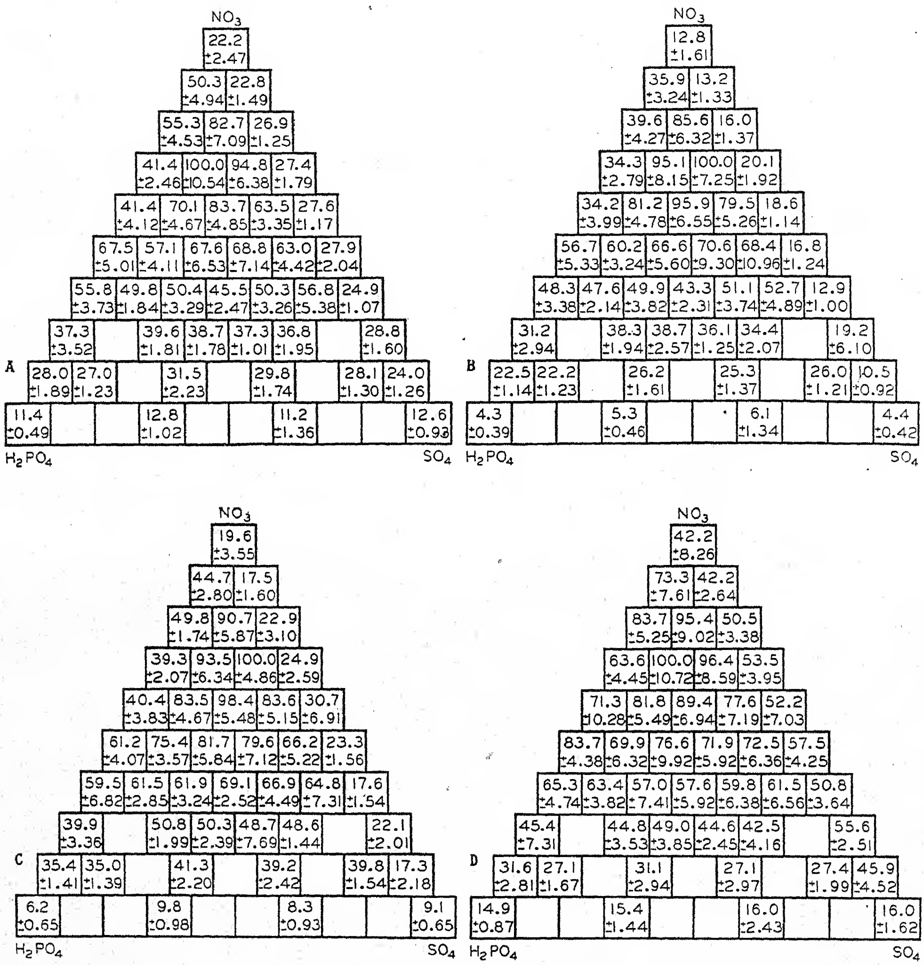

FIG. 5.—Inter-treatment comparison of actual areas of *A*, cortex; *B*, phloem; *C*, xylem; *D*, pith, in stem cross sections. Treatment with maximum value designated 100 and all other treatments related to it. Treatment means, together with standard errors, given.

greater percentage of cortex exists in stem sections from nitrate-deficient treatments, but variability is great, as indicated by the excessively high standard errors involved.

with increments of nitrate. Maximum values are obtained in those treatments supplied with 14.2 m.e./l. Both sulphate-deficient and phosphate-deficient treatments are significantly less than maxi-

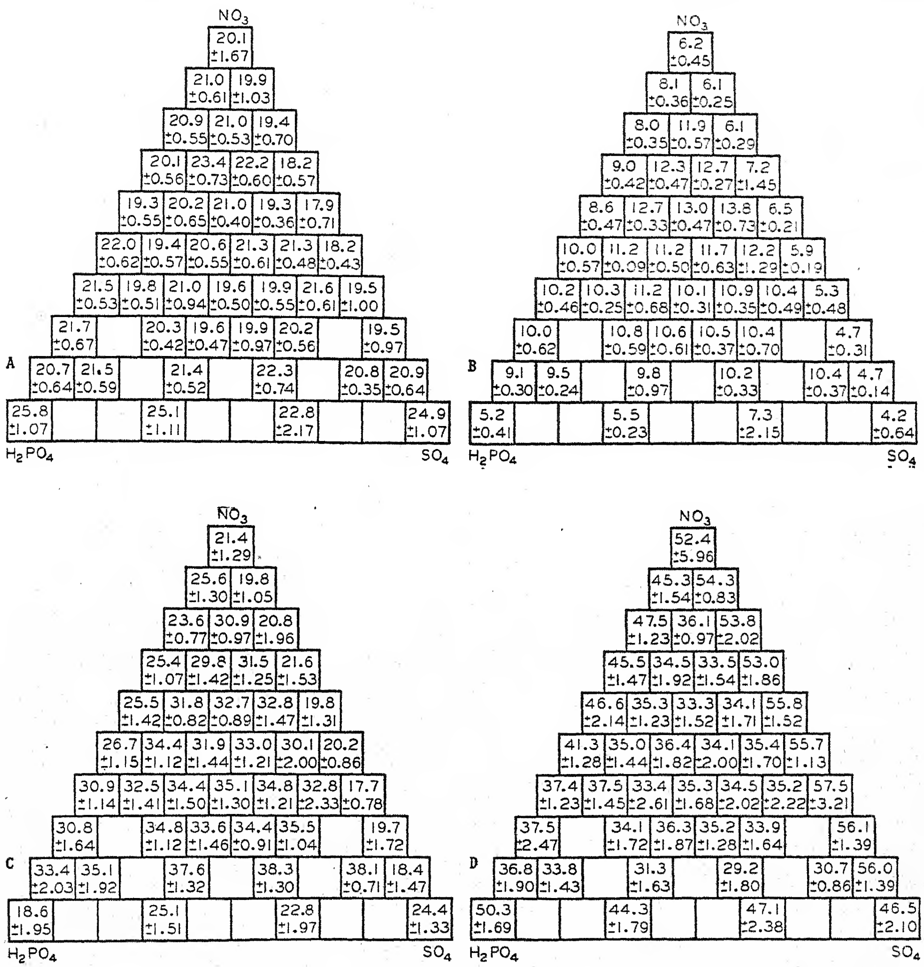

FIG. 6.—Percentage cross-sectional area of stem section occupied by area of *A*, cortex; *B*, phloem; *C*, xylem; *D*, pith. Treatment means, together with standard errors, given.

The percentage cross-sectional area in stem sections occupied by the area of the phloem (fig. 6) shows significant differences which can be correlated with treatment. The relative amount of phloem in stem sections is smallest in nitrate-deficient treatments and increases mum with respect to this characteristic. Thus, not only the actual amount of phloem but the relative amount in each stem section can be correlated with nutrient supply.

The relative amount of xylem in stem sections is smallest in phosphate-defi-

cient but is also small in nitrate-deficient treatments. It is greatest in those treatments receiving only one increment of nitrate in the nutrient medium (2.8 m.e./l.). As greater amounts are supplied to the plant, the relative amount of xylem in the stem section tends to become successively less, although it does not become comparable with values observed for nitrate-deficient stem sections. In sulphate-deficient treatments, a relatively high phosphate and low nitrate supply results in a large relative amount of xylem tissue.

The trends shown in the data for percentage cross-sectional area occupied by pith appear the reverse of those for the relative amount of xylem in stem sections. The greatest relative amount of pith occurs in phosphate-deficient treatments, although the magnitude of the values in nitrate-deficient treatments is also high. The smallest percentage pith was found in stem sections from treatments supplied with 2.8 m.e./l. of nitrate in the nutrient medium. In sulphate-deficient treatments a high nitrate and low phosphate supply results in relatively large amounts of pith.

In these data certain trends seem obvious. Stems from plants supplied with nutrient solutions devoid of nitrate have small amounts of vascular tissue and large amounts of pith. This is also true of phosphate-deficient and to a lesser extent of sulphate-deficient treatments. In treatments supplied with only small amounts of nitrate (2.8 m.e./l.), maximum relative amounts of xylem and minimum relative amounts of pith are present in stem sections. Although the relative amount of phloem tends to increase, the relative amount of xylem in stem sections decreases with increments of nitrate supply. That a unit increase in the relative amount of xylem in a stem section is accompanied by a unit decrease in the relative amount of pith is shown by the highly significant and negative correlation coefficient of -0.971 (table 3). A similar general relationship exists between percentage phloem and percentage pith; and, as would be expected, a significant positive correlation exists between the two components of the vascular system. Since the relative amount of cortical tissue remains constant, no significant correlation with other tissue components of the stem was observed.

One of the most noticeable anatomical differences in the stem sections was the disintegration of central pith cells in all phosphorus-deficient treatments. The remaining pith cells were large, succulent, thin-walled parenchyma with abnormally large intercellular spaces. They were completely devoid of starch. Internal phloem development was at a minimum, with usually three groups of four small sieve tubes and companion cells centrad to the primary xylem points. Both internal and external pericyclic fibers were thin-walled and few in number. Secondary xylem consisted of small thin-walled xylem vessels and wood fibers with undifferentiated parenchymatous cells immediately adjacent to the cambium. Secondary phloem was predominantly parenchyma, and only a few small sieve tubes with companion cells were evident. Cortical parenchyma was similar in appearance to pith parenchyma, with abnormally large intercellular spaces. The development of collenchymatous cells was restricted, and relatively little wall thickening had occurred, especially in those treatments also supplied with small amounts of nitrate. Chlorenchyma was restricted to two or three cell layers immediately internal to the epidermis, and most of these cells had disintegrated.

Central pith cells in nitrogen-deficient

treatments had also disintegrated but less so than in phosphorus-deficient treatments. The parenchymatous pith cells were small in nitrate-deficient treatments and increased in size with increases in nitrate supply. Small accumulations of starch were evident in nitrate-deficient stems immediately centrad to internal phloem, but it did not extend to central pith cells. This resulted in an interrupted ring of starch-filled cells in the outer portion of the pith. The amount of starch accumulated in pith and cortical cells increased as nitrate supply increased, reaching maximum proportions in the central portion of the triangle. Internal pericyclic fibers were practically absent from nitrate-deficient stem sections, but, when present, were extremely small and thick-walled. The number of external pericyclic fibers was not unusual, but they were similar in character to internal fibers. In those treatments receiving 2.8 m.e./l. of nitrate in the nutrient supply, internal and external pericyclic fibers were small but relatively thin-walled, and both size and thickness of wall structure increased with increasing supply. Maximum expression of these characters was noted in treatment no. 5, where 19.8 m.e./l. of nitrate was supplied. Internal phloem formation was negligible in nitrate-deficient stems but increased with increments of nitrate supply, reaching a maximum with respect to number and size of cells in treatment 13 supplied with 14.2 m.e./l. Xylem vessels were small and few in number in nitrate-deficient treatments as contrasted with treatments in the central portion of the triangle. This was the only cellular characteristic of xylem tissue which could be correlated with nitrate supply. Secondary phloem consisted of densely packed small parenchymatous cells in nitrate-deficient treatments, and progressive increases in size and number of cells, as well as differentiation into sieve tubes, companion cells, and phloem fibers were noted with increased nitrate supply. The cortex of nitrate-deficient stems consisted of small parenchyma cells, small relatively thin-walled collenchyma, and a single cell layer of disintegrated chlorenchyma with few chloroplasts. In the central portion of the triangle, the cortex consisted of large parenchyma cells, well-developed and thickened collenchyma, and five or six cell layers of chlorenchyma densely packed with chloroplasts.

TABLE 3

CORRELATION COEFFICIENTS BETWEEN INDICATED VARIABLES IN CROSS SECTIONS OF TOMATO STEMS

ANATOMICAL CHARACTERS	PERCENTAGE		
	Phloem	Xylem	Pith
Percentage*			
Cortex	−0.080	+0.034	−0.196
Phloem	....	+0.812†	−0.870†
Xylem	....	....	−0.971†

* Percentage cross-sectional area of stem section occupied by area of indicated tissue system.

† Highly significant, satisfying statistical requirements for $P = 0.01$.

No distinguishing characteristics of sulphate-deficient stems were observed, their cellular characteristics being influenced to a greater extent by the relative nitrogen and phosphorus supply than by the lack of sulphate as such.

Stem sections obtained from treatment no. 1, supplied with nutrient solutions containing relatively large amounts of nitrate and no phosphate or sulphate, were of interest. Such characteristics as disintegration of central pith cells, large parenchymatous pith cells with abnormally large intercellular spaces, absence of starch accumulation, relatively few

extremely thin-walled internal and external pericyclic fibers, undifferentiated xylem parenchyma adjacent to the cambium, and disintegration of chlorenchyma were found in these stem sections. These characteristics were also present in phosphate-deficient stems. On the other hand, the stem sections were also characterized by thick-walled xylem vessels and wood fibers, relatively numerous sieve tubes and companion cells in the secondary phloem, and abundant thick-walled collenchyma cells in the cortex. All these characteristics were also present in high-nitrate stem sections and were not typical of phosphate-deficiency conditions.

It has been observed that significant and large differences occurred between treatments, not only with respect to stem diameter and the actual area of the component tissue systems, but also with respect to the relative proportions of the tissue systems in the stem. Since stem size is directly correlated with vegetative growth and—to some extent—fruitfulness, it seemed worth while to examine the correlations between actual amounts as well as relative proportions of tissue systems in stem sections with characteristics used as criteria of growth and fruitfulness. The data concerned with growth and fruitfulness of these plants have been previously reported from this laboratory (3, 8). The results of the correlation analysis are given in table 4.

TABLE 4

CORRELATION COEFFICIENTS BETWEEN ANATOMICAL CHARACTERISTICS OF TOMATO STEMS AND CHARACTERS USED AS CRITERIA OF GROWTH AND FRUITFULNESS

ANATOMICAL CHARACTERS	GROWTH				FRUITFULNESS		
	Ht. of vine	Fresh wt. of vine	Dry wt. of vine	Dry wt. of roots	Total no. fruit/vine	Fresh wt. fruit/vine	Ave. fresh wt. mature fruit
Percentage*							
Cortex	−0.129	−0.114	−0.025	+0.001	−0.085	−0.053	−0.167
Phloem	+0.912‡	+0.898‡	+0.910‡	+0.896‡	+0.925‡	+0.913‡	+0.925‡
Xylem	+0.802‡	+0.599‡	+0.615‡	+0.652‡	+0.735‡	+0.681‡	+0.765‡
Pith	−0.827‡	−0.697‡	−0.712‡	−0.739‡	−0.791‡	−0.755‡	−0.797‡
Area†							
Cortex	+0.771‡	+0.932‡	+0.935‡	+0.893‡	+0.830‡	+0.862‡	+0.809‡
Phloem	+0.517‡	+0.967‡	+0.921‡	+0.935‡	+0.875‡	+0.911‡	+0.840‡
Xylem	+0.871‡	+0.977‡	+0.978‡	+0.961‡	+0.930‡	+0.950‡	+0.902‡
Pith	+0.609‡	+0.782‡	+0.784‡	+0.741‡	+0.668‡	+0.693‡	+0.661‡
Stem diameter	+0.827‡	+0.921‡	+0.927‡	+0.901‡	+0.866‡	+0.875‡	+0.864‡

* Percentage cross-sectional area of stem section occupied by area of indicated tissue system.
† Actual area of indicated tissue system in stem cross section.
‡ Highly significant, satisfying statistical requirements for P = 0.01.

These data show stem diameter as well as actual areas of each of the component tissue systems to be significantly and positively correlated with each of the characteristics used as criteria of growth and fruitfulness. This means that each unit increase in the diameter of stems or in the cross-sectional area of tissue systems was accompanied by a unit increase in growth and fruitfulness of the plants. However, the correlation coefficients also indicate that a unit increase in the percentage of cross-section-

al area in stem sections occupied by vascular tissue is accompanied by a unit increase in growth and fruitfulness of the plants. Those correlations involving percentage phloem are equal in magnitude to those involving area phloem, although those involving percentage xylem are significantly less by FISHER's Z test (2) than those involving area xylem. It is also notable that percentage phloem is correlated to a significantly higher degree with growth and fruitfulness than is percentage xylem. The relative proportion of pith is significantly and negatively correlated with growth and fruitfulness, since a strong negative correlation exists between percentage pith and percentage vascular tissue. The relative proportion of cortex is not significantly correlated with any of the characters of growth and fruitfulness, since the percentage cortex remained relatively constant. Thus, in this experiment greater growth and fruitfulness of the plants were accompanied by increased stem diameter, increase in the cross-sectional area of each of the tissue systems, and increase in the relative proportion of vascular tissue in the stem.

Discussion

It seems worth while to consider the foregoing results with respect to nitrate supply in terms of the differences reported by KRAUS and KRAYBILL (6). These investigators characterized stems from feebly vegetative plants grown with a limited nitrogen supply as tough and woody. In a study of their internal structure, they pointed out that pith, cortex, and medullary rays were packed with starch. The walls of outer and inner pericyclic fibers were greatly thickened, while collenchyma development was limited to two or three cell layers. In contrast, they characterized stems from vigorously vegetative plants grown with an abundant nitrogen supply as succulent and brittle. Their diameter was large, as a partial result of the greater number and size of pith cells. No starch had accumulated in pith or cortex, and the relative proportion of xylem in the stem sections was greatly reduced. Both chlorenchyma and collenchyma were well developed, with a large number of chloroplasts in cortical and pith cells.

From this description, it is evident that plants grown with a relatively great nitrate supply in this experiment could be classified as feebly vegetative, owing to the large quantities of starch in pith cells, thick-walled outer and inner pericyclic fibers, and the tough woody nature of the stems. It seems apparent that the high light intensities prevalent during this investigation resulted in extensive carbohydrate synthesis, and that nitrate supply was a limiting factor—even in those treatments supplied with relatively large amounts of nitrate. This supposition is further supported by the fact that fruitfulness increased with increments of nitrate supply and was maximum in those treatments supplied with 17.0–19.8 m.e./l.

A lack of phosphate or sulphate in the nutrient medium was associated with less growth and fruitfulness of tomato plants in this experiment (3). However, a relatively small supply of either ion (2.8 m.e./l.) was sufficient to result in maximum growth and fruitfulness, and no response to successively higher increments in the supply of these ions was obtained. It is apparent from the data presented here that differences in cellular characteristics and tissue system development of tomato stems could be closely correlated with growth and fruitfulness of the plants. While anatomical responses could be correlated with a deficient supply of phosphate or sulphate, the re-

sponses could not be correlated with successive increments of phosphate or sulphate when the supply of either ion was in excess of 2.8 m.e./l.

It is of interest also to compare stem sections from plants grown at Riverside, California, in 1940 (7) with those of the present experiment. A large volume of nutrient containing 8.1 m.e./l. of nitrate was provided for each plant,. and the plants were automatically irrigated with nutrient solution at hourly intervals during the daytime, with one additional irrigation at midnight. Light intensities were comparable with those of the present experiment. The sections were characteristic of vigorously vegetative stems described by KRAUS and KRAYBILL.

In this triangle, maximum growth and maximum fruitfulness were obtained in the same treatment nos. 8, 9, 13, 14. In the cation triangle, however, which involved variations in the supply of calcium, potassium, and magnesium, a somewhat different situation existed. Using criteria similar to those employed in this investigation, maximum vegetative growth was obtained in treatments supplied with relatively small amounts of calcium, while maximum fruitfulness was obtained in treatments supplied with relatively large amounts of calcium (3). Results of anatomical studies in the cation triangle will be presented at a later date and should help in evaluating the correlations observed here. Further discussion of these results will be delayed until the additional data are available.

Summary

1. An inbred strain of Bonny Best tomatoes was grown in sand culture. The effects of forty-four nutrient solutions varying in the relative proportions of macronutrient anions were studied in relation to the anatomy of plant stems. Measurements of stem diameter and the actual area of each of the component tissue systems were recorded. The data were reduced and analyzed by statistical methods.

2. Great differences in stem diameter and the actual area of each of the component tissue systems could be correlated with differences in nutrient supply.

3. Differences in the relative proportion of phloem, xylem, and pith in stem sections were correlated with differences in nutrient supply. No differences were found in the relative amount of cortex.

4. Cellular differences in pith parenchyma, xylem vessels and fibers, internal and external phloem, internal and external pericyclic fibers, and cortical cells are described and correlated with nutrient supply.

5. Differences in the anatomy of the tomato stems are significantly correlated with characteristics used as criteria of vegetative growth and fruitfulness.

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LITERATURE CITED

1. FISHER, R. A., The design of experiments. Oliver & Boyd, London. 1942.
2. ———, Statistical methods for research workers. Oliver & Boyd, London. 1936.
3. HAMNER, K. C., LYON, C. B., and HAMNER, C. L., Factors influencing the ascorbic-acid content of the tomato, with special reference to mineral nutrition. BOT. GAZ. 103:586–616. 1942.
4. HAYWARD, H. E., The structure of economic plants. Macmillan, New York. 1938.
5. HAYWARD, H. E., and LONG, E. M., Anatomical and physiological responses of the tomato to varying concentrations of sodium chloride, sodium sulphate, and nutrient solutions. BOT. GAZ. 102:437–462. 1941.
6. KRAUS, E. J., and KRAYBILL, H. R., Vegetation

and reproduction with special reference to the tomato. Ore. Agr. Coll. Exp. Sta. Bull. 149. 1918.

7. LYON, C. B., Responses of two species of tomatoes and the F_1 generation to sodium sulphate in the nutrient medium. BOT. GAZ. 103:107–122. 1941.
8. LYON, C. B., BEESON, K. C., and BARRENTINE, M., Macro-element nutrition of the tomato plant as correlated with fruitfulness and occurrence of blossom-end rot. BOT. GAZ. 103:651–667. 1942.
9. SNEDECOR, G. W., Statistical methods. Collegiate Press, Ames, Iowa. 1938.
10. TIPPETT, L. H. C., Tracts for computers. Random sampling numbers. Cambridge Univ. Press, London. 1927.
11. YATES, F., The analysis of replicated experiments where the field results are incomplete. Empire Jour. Exp. Agr. 1:129. 1933.

A METHOD OF NUMERICALLY EVALUATING AREAS OF PLANT TISSUE

A. GERALDINE WHITING[1] AND JOHN W. MITCHELL[2]

In order to measure the anatomical characteristics of numbers of plants, a rapid histological schedule resulting in slides showing the tissue areas clearly, and simple methods for measuring regular and irregular areas have been devised. Numerical results obtained by these methods are suitable for statistical analysis.

METHOD.—Samples were collected from specific portions of a plant, such as at given levels of the primary root, the main stem, or at points along a representative branch. These were either sectioned fresh or fixed in a formalin-acetic-alcohol solution and stored for later use. Before sectioning, the preserved material was washed over-night. Sections 40–60μ thick were cut on the freezing microtome. The sections were placed successively in water, aqueous stain, water, then 25% glycerin, and finally mounted in glycerin jelly. The sections were then placed in a 1% aqueous solution of crystal violet for several minutes, according to the intensity of stain desired. In this short time the lignified tissues became deeply stained, while the parenchyma and meristem remained lighter in color. In the case of guayule, the violet also allows contrast with the red-staining Sudan IV, if this stain for rubber is used. The exact timing of each step was not critical, and as the material remained in each solution for only a few moments, the whole process required less than half an hour. Two people working together were able to keep both the cutting and the staining-mounting processes in continuous operation, making ten to twelve finished slides per hour.

For regular areas, such as pith, xylem, and phloem, linear measurements were found most suitable. The image of the section to be measured was projected against a screen which was marked into quarters by two lines crossing at right angles. It was then possible to center the projected image and measure it systematically. Four diameter measurements were made of the pith of the stem. The zones of tissue, such as xylem and bark of the stem, were measured along four radii, one in each quarter of the section. Some selection was necessary in making these measurements because of the irregular pattern of vascular bundles or fiber tissue. The average of the four measurements was used to calculate the

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area of any one tissue. This was done by means of standard formulas for the area of a circle or of the zone between two circles. The ruler used was marked according to the magnification of the image, so that linear measurements were read directly in millimeters and areas were calculated in square millimeters. Reproducibility of the results is illustrated by measurements of tissue areas in three consecutive sections taken from a comparable internode of each of several branches (table 1). In the case of guayule, the symmetrical arrangement of tissues lent itself readily to this type of analysis.

TABLE 1

VARIATION IN TISSUE AREAS OF THREE CONSECUTIVE SECTIONS FROM EACH OF FOUR BRANCHES OF GUAYULE. FIGURES REPRESENT AREAS IN SQUARE MILLIMETERS CALCULATED ON BASIS OF AVERAGE OF FOUR LINEAR MEASUREMENTS

No. of section	Branch no.			
	1	2	3	4
	Pith			
1	0.69	0.55	0.85	0.69
2	0.69	0.58	0.85	0.69
3	0.72	0.53	0.85	0.64
	Xylem			
1	9.71	6.24	5.49	6.85
2	9.83	5.93	5.85	6.47
3	10.15	5.90	5.58	6.53
	Bark			
1	17.49	12.53	11.91	14.51
2	17.57	12.65	12.32	14.24
3	18.55	12.90	12.13	14.24

A totally different problem is encountered in measuring numerous irregular areas, such as bast fibers. A simplified area photometer, manufactured by the American Instrument Company and now commercially available, was used in such cases. The instrument operates on the principle of interception of a light beam focused on a photoelectric cell and measurement of the resulting difference in energy output of the cell in terms of scale divisions.[3]

In order to measure fiber areas, the images were first projected on to a screen over which a circular piece of clear cellulose acetate, 13 inches in diameter, had been fastened. An outline of each fiber group in a representative quarter of a stem was then traced on the acetate sheet, and later these traced areas were filled in with ink. By placing the acetate bearing the inked tracings in the photometer, a reading indicating the amount of light intercepted was quickly obtained, and this reading was later converted into square millimeters of tissue by means of a calibration curve.

In inking the tracings, Roberts' India ink, to which 25% glycerin had been added in amounts of 1 cc. to 10 cc. of ink, was found satisfactory. This mixture was readily washed from the sheets so that they could be used repeatedly. As a precautionary measure, the sheets were tested for constancy and uniformity of light absorption at intervals during their use.

The method for calibrating the instrument was important. To obtain comparable measurements of the known areas used for calibration and the unknown areas of fiber tissue, it was necessary that the instrument be calibrated

[3] MITCHELL, J. W., Measurement of the area of attached and detached leaves. Science 83:334–336. 1936.

with the known areas inked on cellulose acetate, in the same manner as that used in the case of unknown areas. Known areas of suitable sizes were therefore inked on the acetate and read in terms of scale divisions. For convenience, the sizes of these known areas were adjusted to the degree of magnification, so that it was possible to plot a curve with which readings in photometer scale divisions were converted directly into square millimeters of stem tissue. In order to test the reproducibility of results obtained with the photometer, the known areas of different sizes were measured repeatedly, and variation between the replicated readings was found to be relatively small (table 2).

TABLE 2

VARIATION IN REPLICATED PHOTOMETER READINGS OF KNOWN AREAS. READINGS EXPRESSED IN TERMS OF SCALE DIVISIONS

Known magnified area (cm.²)	Equivalent tissue area (mm.²)	Readings			
		1	2	3	4
11.04	0.5	11.8	12.5	11.8	11.9
22.08	1.0	13.4	13.8	13.5	13.6
33.12	1.5	15.3	15.3	15.2	15.2
44.16	2.0	16.7	16.9	16.8	17.2
55.20	2.5	18.2	18.3	18.4	18.5
66.24	3.0	20.0	20.0	20.2	20.2

APPLICATION OF METHOD.—Data from a preliminary study of stem tissues of guayule, *Parthenium argentatum*, illustrate an application of the procedures described. Seedlings of four strains were grown under comparable conditions outdoors. Six months after planting, ten plants from each group were selected for tissue measurements. A cross section was made through the true stem at a level approximately ½ inch above the lowest leaf scars. Area measurements of the tissues were recorded and the data analyzed statistically.

In the case of guayule, it is desirable to have an accurate knowledge of the extent of the bark, those tissues between the vascular and cork cambiums, since this is the major rubber-containing region of the plant. By means of linear measurements, the width and percentage of bark in the stems were determined and the data summarized (table 3). These data indicate that variation in average width and percentage of bark in the cross sections was relatively small, but the percentage of bark in strain B was significantly more than that in plants of strain C, by odds of 19:1. Measurements of the pith, a second rubber-containing tissue, showed that the average percentage area occupied by pith in strain D was 6.9, an amount significantly less than that of strains A and C, which measured 10.9 and 11.1, respectively.

TABLE 3

AVERAGE AMOUNT OF BARK IN CROSS SECTIONS OF STEMS AND PERCENTAGE FIBER IN BARK OF FOUR STRAINS OF GUAYULE PLANTS (TEN PLANTS OF EACH STRAIN)

Strain	Bark		Percentage fiber in bark	
	Width (mm.)	Percentage of entire section	Minimum	Maximum
A	1.39	58.3	18.7	33.8
B	1.31	60.2	10.5	30.8
C	1.31	56.1	24.8	37.2
D	1.29	57.8	16.3	40.2

Measurements of the amount of fiber in the bark of guayule are used to illustrate the application of the method in determining areas of tissues irregular in outline. Quantitative results show that seedling plants varied to a great

extent in amount of fiber, and in some of the plants this nonrubber-producing tissue occupied a relatively large proportion of the total cross-sectional area (table 3, fig. 1).

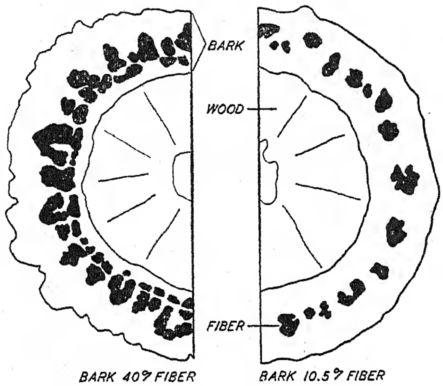

FIG. 1.—Variation in amount of fiber in bark of two plants of two strains of guayule. Plants grown under comparable conditions and sections cut from comparable positions on stem. Diagrams represent tracings of projected images. Fiber areas darkened in a manner similar to that used in making measurements of irregular areas.

In many crop plants, substances of economic importance are produced in special structures, such as rubber in the latex system of *Hevea;* or in specific regions or tissues, such as quinine in the bark of cinchona or fibers in the stems of hemp and flax. Through the application of the method described it is possible to measure anatomical variations which could not be readily determined on the basis of the usual visual method of examination. Furthermore, it permits evaluation of anatomical structures on a quantitative basis useful to the plant breeder in selecting individuals with the most desirable structures and constituents and to the physiologist in determining degrees of structural response to different cultural treatments.

Summary

1. A method of measuring areas of tissues in numbers of plants is described. Sections of the plant were cut with the aid of a freezing microtome, stained, and mounted in glycerin jelly. By means of a projection microscope, an image of the section was thrown on a screen, and areas of tissue symmetrical in outline were calculated from linear measurements, while irregular areas were traced on cellulose acetate, inked, and measured by means of an area photometer.

2. This method is of value in accurately determining the areas or volumes of specific tissues associated with specific functions, such as rubber storage in guayule.

BUREAU OF PLANT INDUSTRY, SOILS, AND AGRICULTURAL ENGINEERING
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WINTER HARDINESS IN JUVENILE AND ADULT FORMS OF CERTAIN CONIFERS[1]

V. R. GARDNER

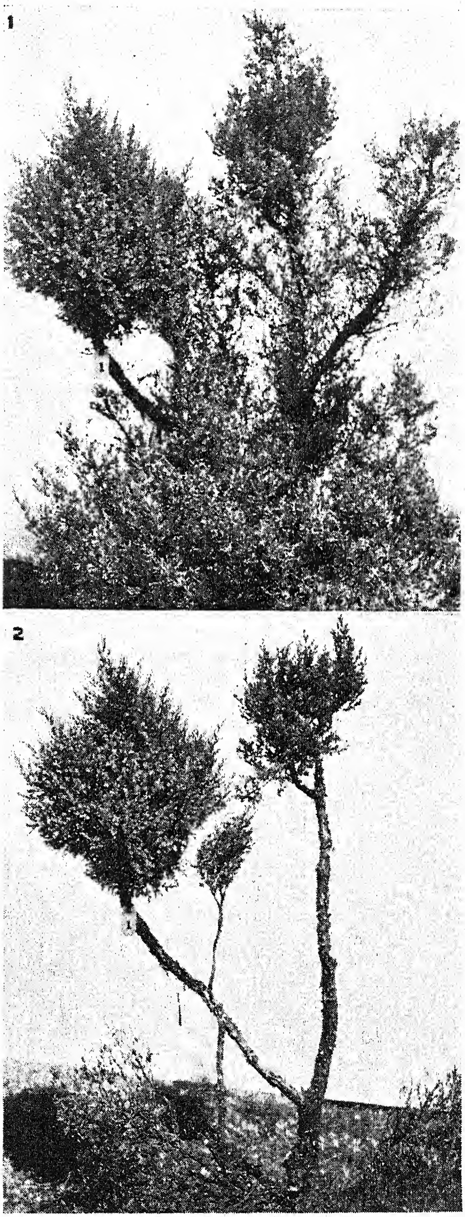
Anatomical and morphological differences between juvenile and adult forms of many conifers have long been noted. Numbers of juvenile and intermediate, as well as of adult, forms have been named, propagated, and introduced into cultivation because of the distinctive characters of their foliage. For the most part these involve shape and color differences of leaves only,

[1] Journal Article no. 669 (n.s.), Michigan Agricultural Experiment Station.

although often associated with such foliage differences are striking variations in general habit of growth. Few observations have been recorded on substantive or physiological differences among these several forms of the same species or on the same plant, although it would seem that—especially in those instances where reversion occurs in individual branches of otherwise normal plants—ideal material would be available for such studies.

Winter injury to a number of plants of *Juniperus chinensis* var. *variegata* and *J. horizontalis* growing on the grounds of the Graham Horticultural Experiment Station near Grand Rapids, Michigan, afforded an opportunity to record some rather striking differences in respect to hardiness. The plants had been set in 1925, had received good care, and had grown well. Those of *J. chinensis* were 6–7 feet tall and had a spread of 4–6 feet in the fall of 1942. Those of *J. horizontalis* were 18–24 inches in height and had a spread of 6–8 feet. Both varieties are characterized by adult appressed, scale-like leaves, but both occasionally produce reverted branches having typical acicular, juvenile-type foliage. These particular plants had produced a number of such reverted branches that were 3–5 years old at the end of the 1942 growing season. The 1942 shoot growth was considerably greater and more vigorous on the juvenile than on the adult portions of the plants and therefore theoretically should have been less mature in the fall.

The winter of 1942–43 was not especially severe, the minimum temperature probably not going below −12° F. Nevertheless many conifers considered winter-hardy in this territory suffered severe killing-back of twigs and branches; in some cases entire plants were destroyed.



FIGS. 1, 2.—Fig. 1, old plant of *J. chinensis* var. *variegata* showing three branches whose terminal portions have reverted to juvenile condition. Entire lower portion and large branch at right have normal adult-type foliage. Fig. 2, plant shown in fig. 1, with winter-killed adult foliage and branches trimmed away.

FIG. 3.—Terminal of old branch (enlarged close-up view of righthand branch shown in fig. 2). Left fork has reverted completely to juvenile condition; right fork shows normal adult-type foliage of the variety, except for several small reverted twigs.

In the spring of 1943 practically all the adult leaves, and the small twigs bearing them, on these two forms of *Juniperus* were dead, while the leaves and wood of the reverted juvenile branches were uninjured. Apparently most of the adult foliage and twigs that survived had had the advantage of some protection by snow. In no instance had the branches bearing juvenile foliage had any advantage of location, either from the standpoint of exposure to minimum temperature or from wind or sunlight. It was clearly a case of a difference in hardiness of tissues between the two forms (figs. 1–3).

In the case of *J. horizontalis*, reverted branches bearing juvenile foliage had struck root more freely where they came in contact with the soil than had branches of the normal form with adult foliage.

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CURRENT LITERATURE

Fundamentals of Cytology. By LESTER W. SHARP. New York and London: McGraw-Hill Book Co., 1943. Pp. x+270. Illustrated. $3.00.

This volume is intended solely as a textbook, and therefore is more than a mere revision of the same writer's well-known *Introduction to Cytology*. Since it is not intended as a work of reference, the author has been free to select for discussion the topics appropriate to an introductory course. Eleven chapters deal with cell structures and functions; five with problems of cytogenetics; and the final chapter with cytology and taxonomy. In harmony with the general plan, the discussion of each topic is brief and so necessarily follows the author's judgment as to what is most important and presents his conclusions in debatable fields. Upon the judgment as to the author's success in these two respects of those teachers who use it, or who consider using it, will obviously depend the ultimate usefulness of the work. The impression of the reviewer is that Professor SHARP has been remarkably successful in both his selection and his discussion. His writing is clear and forceful; recent results are adequately considered, although possibly with a certain tendency to minimize those which are older but still sound; and the conclusions are in general judicious and conservative. A cytologist examining the book may note the absence of some topics he would have included and disagree here and there with the author's views.

All references to literature are omitted in the text, in harmony with the general plan of simplification. The extensive bibliography of the previous work is replaced by a short list for each chapter of discussions—chiefly of a general nature. Terminology has been somewhat simplified. Possibilities here are necessarily limited; so many conceptions are involved in the consideration of scientific results which can be expressed only in technical terms that the demand voiced by many laymen—and an occasional teacher—for a discussion of the results of research in every-day language is a demand for the impossible. Where usage is still inconsistent, a choice of terms must be made; and the author's choice will not in every case be that of the critical reader. It may be asked, for example, why "monoploid" rather than "haploid"; why "kinetochore" instead of "centromere"?

The illustrations, many of them new, are excellently prepared and effectively reproduced.

It is to be hoped that before too long there may be a revision of the author's previous book which, freed now from the limitations of space necessitated by its previous double function, will continue the tradition of an inclusive reference work.—C. E. ALLEN.

Plant Viruses, and Virus Diseases. By F. C. BAWDEN. 2d ed. Waltham, Mass.: Chronica Botanica Co., 1943. Pp. xi+294. Illustrated. $4.75.

The type of the first edition was lost with the invasion of the Netherlands. For this second edition BAWDEN has rewritten more than half the chapters and made various modifications in the others. The most extensive alterations have appeared in discussions of inactivation, of the properties *in vitro*, and of the relationships to insect vectors. Attention is given chiefly to the viruses and relatively little to the virus diseases as such.

The various topics receiving major consideration are similar to those in the first edition, namely: introductory survey; symptomatology; transmission; relationships between viruses and their insect vectors; strains, mutation, and acquired immunity; serological reactions; properties, including optical characteristics; inactivation; sizes of virus particles; physiology of virus-diseased plants; classification of viruses; control of virus diseases; and origin and multiplication of viruses.

Some of the controversies active when the first edition appeared have been resolved and so receive less space. For example, there is now little if any question that the specific protein isolated from an infected plant represents the virus itself. It is accepted that the tobacco mosaic virus is a nucleoprotein instead of a globulin. Concerning controversies still active, the reader will agree or not with BAWDEN, depending on his own view.

The discussion continues as to whether or not a virus is a living entity, but a somewhat different emphasis has been indicated. The present difficulty seems to lie less in our ignorance of viruses than in deciding what criteria to apply to determine whether a substance is alive or not.

The merits of different classifications are still actively debated. Those based upon symptoms have usefulness in certain directions. However, BAWDEN thinks that any lasting system, like those of pathogenic bacteria and fungi, should be based on the intrinsic properties of the viruses themselves and not on those of the host. As all the viruses isolated so far are nucleo-proteins, it is suggested that the methods of classifying proteins may be helpful in making major groups of viruses. Considerable emphasis has been given to the value of serological reactions and other properties.

In relation to mutations, BAWDEN has indicated that some of the evidence is inadequate. The assumption that local lesion technique always gives pure viruses seems to provide a false feeling of security.

Most of the work on plant viruses has been done by pathologists, although in recent years co-operating chemists, crystallographers, entomologists, geneticists, serologists, physicists, and others have been attracted by this intriguing field. While much is known about the viruses themselves, the author finds that little is known about their behavior within the host plant, and this aspect may provide the next major advance. In this direction some valuable methods are already available, which BAWDEN has not discussed. They include, for example, well-known studies of respiratory enzymes and the production of virus-like symptoms (ZIMMERMAN, P. W.,

Contrib. Boyce Thompson Inst. 12:1–14. 1941) with naphthoxyacetic acid and many other known chemicals.

This second edition is a useful and welcome compilation. Since the research on plant viruses has contributed so much to an understanding of their fundamental nature, this book is valuable to the animal as well as to the plant biologist. It is a fine tribute to British appreciation of fundamental science that this excellent work has been continued while bombs were falling.—A. J. RIKER.

An Introduction to Pollen Analysis. By G. ERDTMAN. Waltham, Mass.: Chronica Botanica Co., 1943. Pp. xvi+239. Illustrated. $5.00.

Since its virtual beginning in 1915, the literature of pollen analysis has grown until it now approaches 1700 titles, in many languages. Workers are widely scattered, largely self-taught. From 1927 until 1940, Dr. ERDTMAN has published lists of the literature and maintained close contacts with workers.

The present book presents in a practical and direct way the essentials of technique and procedure. The historical section is brief, and little attempt is made to summarize results, except as they serve to elucidate method. A rather detailed chapter on the chemistry of peat is included for this reason.

The heart of the book consists of six chapters on pollen identification. These, too, are essentially practical in character, rather than "morphological" in the wider sense. Use is made of the methods of description to which WODEHOUSE has made such substantial contribution. The chapter on analysis of tertiary deposits should be of general interest to botanists, as this phase of study is likely to yield increasingly valuable results.

American readers should keep in mind the aim of the book as just set forth. While it is unlikely that they will employ the elaborate symbolism for records and diagrams which has been developed in Europe, it is important that they understand it as presented here. On the other hand, a genetic discussion of peat and related sediments would have been most welcome on this side of the Atlantic, where information of the kind to be found in Vol. 1, *Handbuch der Moorkunde*, is not readily available. BARKLEY'S contribution to the statistical principles of counting probably deserves more than a mere citation.

The difficulties under which this book has been produced would justify far greater limitations than are to be found in it. It is not often that a book with a botanical title can offer so much to workers in so many fields of science. Archaeology, climatology, ecology, floristics, forestry, geology, and limnology will all benefit by those improvements in the method and application of pollen analysis which are the chief concern of this excellent volume.—P. B. SEARS.

The Diagnosis of Mineral Deficiencies in Plants by Visual Symptoms; A Colour Atlas and Guide. By T. WALLACE. London: His Majesty's Stationery Office, 1943. Pp. vi+116. Pls. 114. 10*s*.

This book was written primarily for the use of technical officers and advisors concerned with problems of crop production, for progressive farmers, and for vegetable and fruit growers. It contains elementary discussions of plant nutrition, the ability of soils to supply mineral nutrients to the plant, and methods of determining mineral deficiencies in crops. Of more interest is the discussion of visual symptoms of various mineral deficiencies in horticultural and agricultural crops commonly grown in England. A key is included for the recognition of the most important symptoms in various crops for diagnostic purposes.

The main contribution of the book is the collection of colored photographs, many of which are excellent, although others leave much to be desired. In general, only the gross external appearance of the plant is shown; photographs illustrating individual deficiency symptoms in detail would have been desirable. Nevertheless, the collection in this easily available form, representing the results of the author's experience and study in the field of mineral nutrition, is an important contribution.—C. B. LYON.

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CORRELATION BETWEEN INTERNAL SURFACE AND TRANSPIRATION RATE IN MESOMORPHIC AND XEROMORPHIC LEAVES GROWN UNDER ARTIFICIAL LIGHT

FRANKLIN M. TURRELL

Introduction

The Dalton equation (16), $V = K(F-f)\,\frac{760}{P}\,S$, shows the importance of the area of the water surface (S) in the rate of evaporation (V). TURRELL (21), by means of the internal-external surface ratio (R), showed that the internal surface of leaves from which water evaporates in transpiration is several times larger than the external. If leaf transpiration obeys the same physical laws as evaporation, it is evident from the equation that a leaf with a large internal surface would have a higher transpiration rate under the same conditions than one with a small internal surface, when transpiration rate is based on unit external leaf surface.

The internal surfaces of certain leaves measured by the writer were shown to correlate positively with transpiration rates found by other investigators (20, 21). Since these comparisons and the a priori analyses of the evaporation formula are in general agreement, the investigation reported here was undertaken to determine whether such agreement could be verified by experiment.

Material and methods

Plants of oleander (*Nerium oleander* L.) were selected as a type having thick, xeromorphic leaves and plants of periwinkle (*Vinca rosea* L.) as a type having thin, mesomorphic leaves. In addition to having a normally xeromorphic and a normally mesomorphic type, it seemed possible that a certain degree of xeromorphy could be induced in the mesomorphic and xeromorphic types by growing sets of each under two different light intensities, since it has been shown that leaves exposed to intense sunlight have a greater leaf thickness, palisade thickness, etc., than leaves grown in the shade (3, 12, 8, 10, 14, 17, 5, 6, 9, and others).

Successful growth of plants when employing artificial light has been reported (1, 11, 13, 15, 7, 2). Because of the constant light intensity, ease in regulation of the light period, and ease with which results might be duplicated, the use of artificial light seemed desirable both for growing the plants and for measuring transpiration rates.

Four medium-sized, rooted cuttings of periwinkle and four small rooted cuttings of oleander plants were transplanted to $\frac{1}{2}$-gallon Ball mason jars containing a layer of gravel in the bottom and rich loam soil above. The plants were grown for 2 weeks in these jars under greenhouse conditions to allow for acclimatization. The screw caps of the jars had been previously drilled with three holes, and through one of these holes the plant stem had been inserted in transplanting and sealed with plasticene. Glass tubes were inserted in the other holes and sealed to the cap with plasticene. One tube reached to the jar bottom and one to just above the soil level. The leaves were stripped from the plants at the conclusion of the 2-week

acclimatization period. Two plants of each species were placed under low artificial light intensity and two of each species under high artificial light, in a room specially outfitted for the experiments.

This basement room, in which the plants developed a second complement of foliage and in which the transpiration experiments were conducted, was $9\frac{1}{2}\times12\frac{3}{4}\times8\frac{1}{2}$ feet. It was coated with white cement and was divided into two sections by a wall-board screen. Two hyperbolic galvanized-iron reflectors 18 inches wide, 52 inches long, and 18 inches deep were suspended—one on either side of the screen. Light of high intensity was supplied by three 500-watt Mazda incandescent lamps placed in one of these reflectors and of low intensity by three 200-watt lamps in the other reflector. A wall exhaust fan moved the 981 cubic feet of air in the room at the rate of 600 cubic feet per minute, preventing excessive heating.[1] Despite outdoor summer temperatures as high as 114° F. in the shade, the temperature in the room never exceeded 93° and averaged 87.7° F., according to thermograph records. The relative humidity, which was not controlled, ranged from 46 to 84%. The barometric pressure was steady at 29.5 inches.

When the plants were first placed under the 500-watt lamps, the maximum intensity measured with a Macbeth illuminometer at the table top on which the plants rested was 300.7 foot-candles ("high intensity") and under the 200-watt lamps was 85.9 foot-candles ("low intensity"). Twenty-eight inches above the table top the high intensity (500-watt) measured 964.5 foot-candles, while under the low intensity (200-watt) it measured 300.4 foot-candles. During the transpiration experiment the intensity averaged 175.0 foot-candles on the table top under the 500-watt lamps and 76.8 foot-candles under the 200-watt lamps. These ranges are far below those of summer noon sun.

During the 60 days in which the plants were allowed to develop new foliage, they were exposed to 12-hour photoperiods per day under the artificial light. All the plants made vigorous growth, and the periwinkles under the high intensity blossomed. At this time it was assumed that the plants had developed some mature foliage and that sufficient differentiation had taken place in the structure of the leaves to affect the transpiration rates.

The plants were then well watered, and the glass watering tubes through the jar cap—which had remained uncorked during the growth period—were corked. The plant potometers were weighed on a double-beam agate-bearing balance having a capacity of 5000 gm.[2] Weighings were then made each morning and evening to determine the water lost by transpiration. This amount was replaced after each light period.

Three experiments were conducted. In the first the transpiration of both periwinkle and oleander plants was measured under the same light intensity in which they were grown. Two 250-cc. beakers, filled to within $\frac{1}{2}$ inch of the brim with distilled water, were placed—one in each intensity—and the water loss by evaporation measured for the same period as for transpiration. Using the

[1] About 3% of radiation of the Mazda lamp is in the visible (19).

[2] Under a load of 2200 gm. the sensitivity of the balance was 0.48 gm./division, while under a load of 3800 gm. it was 1.0. The heaviest potometer weighed about 3600 gm., and the average transpiration loss was 100 gm. between two subsequent weighings. Thus transpiration loss could be measured with an error of not greater than ± 1.0%.

difference in the amount of water evaporated from the beakers as a basis, a correction factor was applied to the transpiration rate of the plants to compensate for the water loss due to the greater radiation in the high intensity. Transpiration and evaporation losses were measured over approximately a 3-day period.

In the second experiment periwinkle and oleander plants with foliage grown under high intensity were placed in low intensity together with similar plants with foliage grown in low intensity. Transpiration and evaporation losses were measured over approximately a 3-day period.

The first and second experiments were conducted in the order given and prior to the third experiment, in order that as little change as possible might be induced in the structure of the immature leaves. For the third experiment, periwinkle and oleander plants with foliage grown in high intensity and with foliage grown in low intensity were all placed under high intensity. Transpiration and evaporation were measured over approximately a 3-day period.

On the conclusion of the transpiration experiments, sections of tissue 0.25 sq. cm. were cut from the center of the blades of an upper and lower leaf of each of the plants and killed in formalin-acetic acid-alcohol solution. From these sections permanent microscope slides were prepared, internal surface measurements made according to the method described by TURRELL (21), and the ratio (R) of the internal surface per unit external surface was determined.

Immediately after selection of the sections for the internal surface measurements, the leaves were stripped from each plant and blueprints made of the leaves with a Daylight carbon-arc lamp. The areas of the leaves were subsequently measured from the blueprints with a planimeter.

The results of the three experiments are not mutually comparable, since the transpiration data have not been reduced to the same number of hours for each of the experiments although the same plants have been used in each.

Results

NATURAL AND INDUCED XEROMORPHY.—The foliage grown in high intensity was more xeromorphic in both periwinkle and oleander than that grown in low intensity (table 1). The foliage of oleander was more xeromorphic than that of periwinkle, regardless of the intensity of light in which it was grown (table 1).

The differences of leaf and tissue thicknesses of the leaves grown in high and in low intensity are shown in table 2. Comparison of the ratios for tissue and leaf thicknesses in both plants shows the relative xeromorphy induced. Table 2 indicates that high intensity induced a relatively greater xeromorphy in periwinkle than in oleander leaves—1.11 times, judging on the basis of leaf thickness, and 3.00 times, judging on the basis of palisade thickness. The effect of high intensity, however, was relatively less (0.73 times) on the thickness of the upper epidermis of the periwinkle than on that of the oleander leaves.

The greater induced xeromorphy in the foliage of the plants grown under high intensity and the greater natural xeromorphy of the oleander plants are reflected in the internal-external surface ratios (R). An average of these ratios for upper and lower leaves of each plant is shown in table 3a. Leaves of both species grown in high intensity had higher internal-external surface ratios than when grown in low intensity. (One-

hundred-sixty determinations of the effect of light intensity on (*R*) were made for periwinkle, and also for oleander leaves, by a modification of a previous method (23).)

STOMATAL SIZE AND INTERSTOMATAL DISTANCES.—Leaves of periwinkle had stomata more or less regularly distributed on the upper and lower surfaces. The stomata averaged 22.2 μ in length and 14.1 μ in width—including the guard cells. The stomatal pore length averaged 11.4 μ and the width 2.3 μ. In large leaves the interstomatal-pore distance averaged 70.0 μ and in small leaves 61.2 μ. Leaves of oleander had no stoma-

TABLE 1

CHARACTERISTICS OF LEAVES OF PLANTS GROWN UNDER DIFFERENT LIGHT INTENSITIES (THREE LAMPS USED)

Plant	Lamp wattage	Light intensity	Leaf thickness (μ)	Palisade depth (μ)	Thickness of upper epidermis (μ)	Thickness of upper cuticle (μ)
Periwinkle 1, 2	500	High	130	37	21	<1.0
Periwinkle 3, 4	200	Low	108	25	15	<1.0
Oleander 1, 2	500	High	239	64	48	3.5
Oleander 3, 4	200	Low	203	55	31	1.8

TABLE 2

RELATIVE XEROMORPHY DEVELOPED IN PLANTS BY HIGH LIGHT INTENSITY

Plant	Leaf thickness (high light−low light)	Palisade thickness (high light−low light)	Upper epidermal thickness (high light−low light)
Periwinkle	22μ	12μ	6μ
Oleander	36	9	17
	$\frac{\text{Diff. in leaf thickness}}{\text{Leaf thickness}}$	$\frac{\text{Diff. in palisade thickness}}{\text{Palisade thickness}}$	$\frac{\text{Diff. in epidermal thickness}}{\text{Epidermal thickness}}$
Periwinkle	0.20	0.48	0.40
Oleander	0.18	0.16	0.55
	$\frac{\text{Diff. in leaf thickness/leaf thickness}}{\text{Diff. in leaf thickness/leaf thickness}}$	$\frac{\text{Diff. in palisade thickness/palisade thickness}}{\text{Diff. in palisade thickness/palisade thickness}}$	$\frac{\text{Diff. in epidermal thickness/epidermal thickness}}{\text{Diff. in epidermal thickness/epidermal thickness}}$
$\frac{\text{Periwinkle}}{\text{Oleander}}$	1.11	3.00	0.73

All oleander leaves had greater (*R*) than periwinkle leaves, regardless of the intensity in which they were grown (table 3a). Analysis of variance has shown that the differences in these three comparisons are all highly significant (table 3b).

ta on the upper leaf surface, while on the lower surface they were crowded together in pits sunk in the lower epidermis, and covered with hairs. The stomata averaged 21.8 μ in length and 15.4 μ in width—including the guard cells. The stomatal pore length averaged 8.0 μ and the width 2.6 μ. The interstomatal-pore distance was 29.3 μ and the interstomatal-pit distance was 113.0 μ.

TABLE 3a

AVERAGE INTERNAL-EXTERNAL SURFACE RATIOS (R) FOR UPPER AND LOWER LEAVES GROWN UNDER DIFFERENT LIGHT INTENSITIES

Grown under high intensity		Grown under low intensity	
Plant	(R)	Plant	(R)
Periwinkle 1...	8.86	Periwinkle 3...	8.34
Periwinkle 2...	8.59	Periwinkle 4...	6.57
Oleander 1.....	14.90	Oleander 3.....	13.35
Oleander 2.....	18.85	Oleander 4.....	15.10

Experiment I: Transpiration measured simultaneously in low and in high light intensity

A. The transpiration losses in high intensity of two periwinkle and two oleander plants, with foliage grown under high intensity, are shown in table 4. These losses, reduced to grams of water transpired per cm.[2] of leaf surface, are shown in table 5. If the length of the experimental period is considered as a unit time, the values shown are transpiration rates.

The transpiration rate of oleander was greater than that of periwinkle when measured in high intensity (table 5). The (*R*) was greater in the oleander plants having foliage grown in high intensity than similarly grown foliage of periwinkle (table 3a). The calculated coefficient of correlation (*r*) between the transpiration rate and (*R*) is +0.787 (table 6). This value is high and positive but not sufficiently high for 2 degrees of freedom to be significant.

B. Simultaneously, and for the same length of time as in A, transpiration losses in low intensity of the two periwinkle and two oleander plants, having foliage grown in low intensity, were measured (table 4). The transpiration rates were greater in oleander than in periwinkle (table 5). The (*R*'s) were also greater for oleander (table 3a). The coefficient of correlation between the transpiration rate and (*R*) is +0.744 (table 6). This value is high and positive but not sufficiently high for 2 degrees of freedom to be significant.

TABLE 3b

SUMMARY OF ANALYSIS OF VARIANCE OF (R)

Source of variation	Degrees of freedom			Sum of squares			Mean square		
	Periwinkle vs. oleander	Periwinkle high vs. low light	Oleander high vs. low light	Periwinkle vs. oleander	Periwinkle high vs. low light	Oleander high vs. low light	Periwinkle vs. oleander	Periwinkle high vs. low light	Oleander high vs. low light
Total..............	7	159	159	130.87	386.99	1545.75	18.68	....	....
Between...........	1	1	1	111.30	81.76	302.37	111.30*	81.76*	302.37*
Within............	6	158	158	19.59	305.23	1243.38	3.26	1.93	7.87

* Highly significant.

C. The total loss of water from the beakers as evaporation in high and in low intensity, measured simultaneously and for the same length of time as the transpiration of the plants in A and B, was 106 and 88 gm., respectively (table 7). Reducing these values to evaporation per unit area of free water surface gives 1.70 and 1.42 gm./cm.²/period for evaporation in high and in low intensity, respectively. The difference in the rate of evaporation (19.7%) in the two beakers is due primarily to the difference in intensity of the radiation incident upon the water in them. The transpiration rate values of periwinkle plants measured in high intensity were reduced 19.7% to equalize the effects of light intensity.

After making these equalizations, the correlation coefficient between (R) and the transpiration rate of periwinkle plants with foliage grown in high intensity and the transpiration measured in high and of such plants with foliage grown in low intensity and the transpiration measured in low was +0.996 (table 6). This value is high, positive, and highly significant.

D. The transpiration rate values of oleander plants measured in high in-

TABLE 4

EFFECT OF LIGHT INTENSITY ON TOTAL TRANSPIRATION OF PLANTS GROWN UNDER HIGH AND LOW INTENSITIES*

Plant	Total upper and lower leaf surface (cm.²)	Transpiration			
		Experiment I		Experiment II	Experiment III
		Low intensity (gm.)	High intensity (gm.)	Low intensity (gm.)	High intensity (gm.)
	Grown under high intensity				
Periwinkle					
No. 1	2720	....	549	639	531
No. 2	1890	....	334	428	300
Oleander					
No. 1	551	....	261	430	415
No. 2	414	....	150	297	268
	Grown under low intensity				
Periwinkle					
No. 3	1920	241	....	459	381
No. 4	616	55	....	91	51
Oleander					
No. 3	547	125	....	454	409
No. 4	538	82	....	437	327

* Duration of experiments I, II, and III is not the same, thus the transpiration losses per experimental period are not mutually comparable.

tensity were reduced 19.7% to equalize the effects of radiation on transpiration losses. Despite this reduction, the rates for oleander with foliage grown in high and transpiration measured in high and the transpiration when grown in low and transpiration measured in low are correlated with internal surface only to a moderate degree. The correlation coefficient of +0.428 is not statistically significant.

E. After applying the correction factor, if all eight plants in this experiment are considered together in calculating the correlation coefficient between transpiration rate and (R), the value of +0.852 is high and highly significant.

TABLE 5

EFFECT OF LIGHT INTENSITY ON TRANSPIRATION RATES OF PLANTS GROWN UNDER HIGH AND LOW INTENSITIES*

PLANT	TRANSPIRATION			
	Experiment I		Experiment II	Experiment III
	Low intensity (gm./cm.²/period)	High intensity (gm./cm.²/period)	Low intensity (gm./cm.²/period)	High intensity (gm./cm.²/period)
	Grown under high intensity			
Periwinkle				
No. 1	....	0.202	0.235	0.195
No. 2	....	0.177	0.226	0.159
Oleander				
No. 1	....	0.474	0.781	0.754
No. 2	....	0.362	0.718	0.647
	Grown under low intensity			
Periwinkle				
No. 3	0.125	....	0.239	0.198
No. 4	0.090	....	0.148	0.083
Oleander				
No. 3	0.228	....	0.829	0.748
No. 4	0.152	....	0.814	0.608

* Duration of experiments I, II, and III is not the same, thus the transpiration rates per experimental period are not mutually comparable.

EXPERIMENT II: TRANSPIRATION MEASURED IN LOW LIGHT INTENSITY

Plants of both species grown in high intensity were placed in low together with both types grown in low, and transpiration of all plants was measured. The total transpiration losses are shown in table 4, and the losses per cm.²/period are given in table 5.

A. A high correlation existed between (R) and transpiration rate in periwinkle. The (r) was +0.941, but it is of doubtful significance as it is below the 5% level although above the 10% level.

B. In the oleander plants, (r) was

TABLE 6

CORRELATION COEFFICIENTS FOR RELATION OF TOTAL TRANSPIRATION OF PLANTS GROWN UNDER DIFFERENT LIGHT INTENSITIES AND MEASURED UNDER SAME OR DIFFERENT INTENSITIES, AND INTERNAL-EXTERNAL SURFACE RATIO (R)

Exp. no. and comparison designation	Plant		Correlation coefficient of transpiration			Degrees of freedom	Significance†
	Grown under high intensity	Grown under low intensity	High intensity	Low intensity	Intensity in which grown		
I. A.	Periwinkle, Oleander	.....	+0.787	.....	.....	2	Ns
I. B.	.....	Periwinkle, Oleander	.....	+0.744	.....	2	Ns
I. C.	Periwinkle*	Periwinkle	.....	.....	+0.996	2	Hs
I. D.	Oleander*	Oleander	.....	.....	+0.428	2	Ns
I. E.	Periwinkle, Oleander*	Periwinkle, Oleander	.....	.....	+0.852	6	Hs
II. A.	Periwinkle	Periwinkle	.....	+0.941	.....	2	G
II. B.	Oleander	Oleander	.....	−0.953	.....	2	S
II. C.	Periwinkle, Oleander	.....	.....	+0.915	.....	2	G
II. D.	.....	Periwinkle, Oleander	.....	+0.988	.....	2	S
II. E.	Periwinkle, Oleander	Periwinkle, Oleander	.....	+0.893	.....	6	Hs
III. A.	Periwinkle	Periwinkle	+0.940	.....	.....	2	G
III. B.	Oleander	Oleander	−0.538	.....	.....	2	Ns
III. C.	Periwinkle, Oleander	.....	+0.890	.....	.....	2	Ns
III. D.	.....	Periwinkle, Oleander	+0.946	.....	.....	2	G
III. E.	Periwinkle, Oleander	Periwinkle, Oleander	+0.890	.....	.....	6	Hs

* Corrected for evaporation caused by difference in radiation intensity.

† Ns = not significant; G = above 10% level; S = above 5% level (significant); Hs = above 1% level (highly significant).

TABLE 7

EFFECT OF DIFFERENT LIGHT INTENSITIES ON EVAPORATION OF WATER IN CONTAINERS (FREE WATER SURFACE)

Free water surface	Surface area (cm.²)	Evaporation							
		Experiment I				Experiment II		Experiment III	
		Low intensity		High intensity		Low intensity		High intensity	
		gm./period	gm./cm.²/period	gm./period	gm./cm.²/period	gm./period	gm./cm.²/period	gm./period	gm./cm.²/period
No. 1	62.1	.....	.....	106	1.70	46	0.743	100	1.61
No. 2	62.1	88	1.42	.....	.....	47	0.759	100	1.61

-0.953, which is high, negative, and significant (table 6).

C. When both the periwinkle and oleander plants grown in high intensity are considered, the correlation between (R) and transpiration rate is high and positive ($r = +0.915$), but it is only significant at the 10% level (table 6).

D. When both the periwinkle and oleander plants grown in low intensity are considered, the correlation coefficient between (R) and transpiration rate is high, positive ($r = +0.988$), and significant (table 6).

E. The best test of the relation between (R) and transpiration rate results from consideration of all the plants together, regardless of the light intensity in which they were grown. In this case the (r) is $+0.893$, which is high, positive, and highly significant—above the 1% level (table 6).

Experiment III: Transpiration measured under high light intensity

On completion of experiment II, all the plants (both periwinkle and oleander grown in high and low intensity) were placed in high and their transpiration measured. Total transpiration losses are shown in table 4 and losses per cm.²/period in table 5.

A. A high correlation existed between (R) and transpiration rate in periwinkle. The (r) was $+0.940$, but it is of doubtful significance as it is below the 5% level although above the 10% (table 6).

B. In oleander the (r) was -0.538, which is moderate, negative, and not significant (table 6).

C. When both the periwinkle and oleander plants grown in high intensity are considered, the correlation between (R) and transpiration rate is high and positive ($r = +0.890$), but it is just below the point of significance at the 10% level (table 6).

D. When both species grown in low intensity are considered, the correlation coefficient between (R) and transpiration rate is high, positive ($r = +0.946$), and of doubtful significance, as it is just below the 5% level and above the 10% (table 6).

E. As in experiments I and II, the best test of the relation between (R) and transpiration rate results from consideration of all the plants, regardless of the light intensity in which they were grown. In this case the correlation coefficient is $+0.890$, which is high, positive, and highly significant (table 6).

Relation of stem transpiration to total transpiration

To determine the maximum error introduced by treating total transpiration as leaf transpiration, the defoliated stems of all the plants used in these experiments were placed in high intensity and illuminated continuously for 11 days. A fan was turned directly on the plants to induce as great water loss as possible. Under these conditions the average transpiration per day was 1.34 gm. per stem, as compared with approximately 100 gm. per day per normal plant. Thus the maximum error in using total transpiration as leaf transpiration is approximately 1.34%.

Discussion

The experiments as conducted and the conclusions drawn from them are based on several assumptions: (*a*) that the roots of the plants were in an active and healthy growing condition, so that they were able to absorb water readily; (*b*) that the soil contained ample water, easily to supply all needs of the plants; and (*c*) that the evaporating conditions

for the tops were not so great that the rate of water absorption by the roots was a limiting factor. It was concluded that these assumptions were reasonably correct, for the root systems were well developed and no dead roots were found at the conclusion of the experiment; the plants were well watered during the experiments and the soil in the potometers moist at their conclusion; and no wilting of the leaves was observed at any time.

The effect of stomatal control over transpiration was not measured, but the effects so far as these experiments were concerned were minimized as much as possible by selecting two genera with fundamentally different stomatal distributions. Also, the use of plants grown in different light intensities but with transpiration measured under the same intensity tends to minimize the effect of stomata, for similar stomatal distributions permit water vapor diffusion from different-sized areas of internal surface.

Using SAYRE's modification (18) of BROWN and ESCOMBE's law (4), it was readily calculated that the stomata in the leaves of periwinkle were 2.5 times too close together to secure maximum diffusion in still air; in the stomatal pits of oleander it was 5.74 times. In the latter, when the pits were considered as a unit, the stomatal groups were still 1.49 times too close for maximum diffusion.

Diffusion is proportional to pore area in moving air (4). In the periwinkle leaves the area of a stomatal pore averaged 81.3 μ^2 and in the oleander 65.5 μ^2. The stomatal pits, located on only one side of the oleander leaves, contained eight to ten stomata per pit. The stomata in the periwinkle leaves were located on both leaf surfaces, but it was estimated that the oleander leaves would suffer less interference with diffusion in moving air. They have approximately 184,000 μ^2 of pore area per mm.^{2} of leaf area, while periwinkles have approximately 110,000 μ^2 of pore area. These figures suggest that a positive correlation may exist between stomatal pore area per mm.^{2}, internal leaf surface, and transpiration rate.

Stem transpiration—although appreciable—was too small to influence significantly the results of leaf transpiration. The error in the measurement of transpiration rate likewise was small. The internal surface measurements were extremely accurate for the sample taken. However, the small number of samples which could be analyzed undoubtedly made the sampling error large. Two other factors—the horizontal and the vertical light distribution as described by the inverse square law—had an effect on the morphological structure of the leaves. This has been discussed (22), and it may suffice here to say that artificial light is excellent for many types of experimental work with plants, but it does not provide a good light source for an experiment of this kind (22). Three to four per cent of the radiation of a Mazda lamp is in the visible range (19), and this quality of the lamp may also have influenced the structure and behavior of the plants to some extent.

My interpretation of the significant correlation between the transpiration rates and internal-external surface ratios of periwinkle leaves developed under the two intensities, when measured under two intensities in the first experiment, is that a very significant degree of xeromorphy had been induced by the high intensity under 500-watt lamps (table 2). Other correlations, in which four oleander plants were used alone or two oleander with two periwinkle plants, were not significant in the first experiment because only a small (although signifi-

cant) degree of xeromorphy was developed in the oleander leaves (table 2). This fact, together with the limited number of degrees of freedom available, was sufficient to reduce the correlation coefficients to nonsignificance, although they were positive. In the correlations based on all the plants the natural xeromorphy and high transpiration rates of the oleander leaves contributed considerable weight, and with the larger number of degrees of freedom gained, gave correlation coefficients which were high, positive, and highly significant (table 6).

In experiments II and III the correlation coefficients between transpiration rate and (R) are high and significant at the 5% level, 10% level, or are nearly significant when four plants are compared (except for oleander plants alone). The improvement in the correlations in the latter experiments over the first is probably dependent on the fact that all the plants were under the same light intensity, and that the limitations of a correction factor for different intensities did not enter into the calculations. When all the plants were used in calculating a correlation coefficient between (R) and transpiration rate, (r) was high, positive, and highly significant.

No investigation was made of the causes underlying the high negative and significant correlation in experiment II, where the transpiration rates of oleander plants were measured under low intensity, and there seems no complete explanation for it. Likewise, in experiment III the same comparison under high intensity gives a lower but nonsignificant negative correlation coefficient. Several factors are probably involved, however, among which are the effect of light intensity and stomatal closure under low intensity in the leaves which had been grown under high intensity.

In experiment II, when transpiration rate of periwinkle plants is compared with evaporation from a free water surface, the transpiration/evaporation ratio was 0.28, and in oleander it was 1.05. In experiment III the ratios were 0.10 and 0.43 for periwinkle and oleander, respectively. While the absolute values for transpiration are not comparable in the two experiments, the relative rates of transpiration to evaporation are. Calculation shows that the periwinkle plants reduced their relative transpiration/evaporation ratio under high intensity 64.3% and the oleander plants 59.1%. Both experiments indicate the efficiency of the leaf in conserving water, for the internal transpiring surface of the periwinkle leaves per $cm.^2$ of leaf area averaged 16.18 times the unit free water surface (1 $cm.^2$), and the internal transpiring surface of oleander per $cm.^2$ of leaf area averaged 31.10 times. These calculations also suggest that the more drastic the evaporating conditions, at least over a certain range, the more efficient the leaf mechanism becomes in conserving water as compared with a free water surface.

The actual efficiency of the leaf can be more readily appreciated when it is recalled that the internal transpiring leaf surface in periwinkle averaged 8.1 times the external surface, and in oleander, 15.6. For example, in experiment III the actual ratio of water evaporated (transpired) from the internal leaf surface per $cm.^2$/period to the evaporation of water from a free water surface per $cm.^2$/period is 0.012 for periwinkle and 0.027 for oleander. These calculations show that the transpiration is much less per unit leaf internal surface than evaporation from a free water surface. They also show that the oleander leaves transpired on the average more than twice as much per unit area of internal surface as the peri-

winkle, indicating the existence of leaf differences in the two genera other than area of internal leaf surface. These differences may be in part morphological, but most likely they are physico-chemical (for example, osmotic pressure).

The experiments indicate that the size of the area of internal exposed surface per unit external leaf surface is strongly correlated with transpiration rates per unit area, as might be surmised from examination of the Dalton equation (16) or other equations for the physical evaporation of water. Other factors also are shown to be partially responsible for the high correlation of internal leaf surface and transpiration rate.

Summary

1. Plants of periwinkle and oleander were grown under two ranges of intensity of artificial light. The low intensity range was 86–300 foot-candles and the high intensity range was 301–965 foot-candles. Both the mesomorphic leaves of periwinkle and the xeromorphic leaves of oleander developed a significant degree of xeromorphy under high intensity, when judged on the basis of the relative increase in palisade thickness.

2. The xeromorphic leaves of oleander had significantly greater internal-external surface ratios than the mesomorphic leaves of periwinkle. The leaves of both species grown under high intensity had significantly larger internal-external surface ratios than the leaves of those grown under low intensity.

3. Transpiration rates of both species were measured by weighing in potometers. When all the plants were treated statistically as a group, correlations between the internal-external surface ratios and the transpiration rates were high and highly significant ($r = +0.85$ to $+0.89$). These correlations obtained, whether transpiration was measured under low (minimum 77 foot-candles), under high (minimum 175), or under the light intensity in which the plants were grown, when in the latter instance correction was made for radiation. Individual comparisons, however, were often not significant.

4. The xeromorphic leaves of oleander had greater transpiration rates than the mesomorphic leaves of periwinkle per unit external leaf surface, as predicted from evaporation equations for free water surfaces; but computations of transpiration per unit internal leaf surface indicated other factors were also responsible for the greater transpiration rate in xeromorphic leaves.

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LITERATURE CITED

1. Arthur, J. M., Guthrie, J. D., and Newell, J. M., Some effects of artificial climates on the growth and chemical composition of plants. Amer. Jour. Bot. **17**:416–482. 1930.
2. Arthur, J. M., Weight of plant tissue produced in relation to the efficiency of an incandescent filament lamp. Amer. Jour. Bot. **19**:848–849. 1932.
3. Bergen, J. Y., Transpiration of sun leaves and shade leaves of *Olea europaea* and other broad-leaved evergreens. Bot. Gaz. **38**:285–296. 1904.
4. Brown, H. T., and Escombe, F., Static diffusion of gases and liquids in relation to the assimilation of carbon and translocation in plants. Roy. Soc. Phil. Trans. London Ser. B **193**:223–291. 1900.

5. CAIN, S. A., and MILLER, J. D. O., Leaf structure of *Rhododendron catawbiense* Michx. Amer. Mid. Nat. 14:69–82. 1933.
6. CAIN, S. A., and POTZGER, J. F., A comparison of leaf tissues of *Gaylussacia baccata* (Wang.) C. Koch and *Vaccinium vacillans* Kalm. grown under different conditions. Amer. Mid. Nat. 14: 97–112. 1933.
7. DAVIS, A. R., and HOAGLAND, D. R., An apparatus for the growth of plants in a controlled environment. Plant Physiol. 3:277–292. 1928.
8. DOI, T., Über die Sonnen- und Schattenblätter einiger Bäume. Imp. Univ. Jour. Col. Sci. Tokyo 40:1–36. 1917.
9. DUNCAN, W. H., Ecological comparison of leaf structures of *Rhododendron punctatum* Andr. Amer. Mid. Nat. 14:83–96. 1933.
10. HANSON, H. C., Leaf-structure as related to environment. Amer. Jour. Bot. 4:533-560. 1917.
11. HARVEY, R. B., Growth of plants in artificial light. BOT. GAZ. 74:447–451. 1922.
12. HASSELBRING, H., The effect of shading on the transpiration and assimilation of the tobacco plant in Cuba. BOT. GAZ. 57:257–286. 1914.
13. HENDRICKS, E., and HARVEY, R. B., Growth of plants in artificial light. BOT. GAZ. 77:330–334. 1924.
14. KISSELEW, N. N., Über die Transpiration welkender Sonnen- und Schattenblätter. Bot. Centralbl. Beihefte 44:181–217. 1927.
15. MAXIMOV, N. A., Pflanzenkultur bei elektrischen Licht und ihre Anwendung bei Samenprüfung und Pflanzenzüchtung. Biol. Zentbl. 45:627–639. 1925.
16. ———, The plant in relation to water. Transl. by YAPP. Allen and Unwin, London. 1929.
17. PENFOUND, W. T., Plant anatomy as conditioned by light intensity and soil moisture. Amer. Jour. Bot. 18:558–572. 1931.
18. SAYRE, J. D., Physiology of stomata of *Rumex patientia*. Ohio Jour. Sci. 26:233–267. 1926.
19. SHIRLEY, H. L., Light sources and light measurements. Plant Physiol. 6:447–466. 1931.
20. TURRELL, F. M., The internal exposed surface of foliage leaves. Science 78:536–537. 1933.
21. ———, The area of the internal exposed surface of dicotyledon leaves. Amer. Jour. Bot. 23:255–264. 1936.
22. ———, The relation between chlorophyll concentration and the internal surface of mesomorphic and xeromorphic leaves grown under artificial light. Iowa Acad. Sci. Proc. 46:107–117. 1939.
23. ———, A quantitive morphological analysis of large and small leaves of alfalfa, with special reference to internal surface. Amer. Jour. Bot. 29:400–415. 1942.

EFFECTS OF VARIATION IN NUTRIENT SOLUTION ON GROWTH OF SUNFLOWER PLANT

CONTRIBUTIONS FROM THE HULL BOTANICAL LABORATORY 559

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Introduction

For some time the writer has been studying the effects of sulphur deficiency on the growth of plants, and a summary paper has recently been published (4). In much of this work, nutrient solution IR2S4 of the LIVINGSTON triangle (11) was used, since preliminary work indicated that it was best for growth of the sunflower. Others have used this solution (13). In the sulphur-deficiency work on the mustard (4), this solution was diluted somewhat, since growing the plant at various dilutions indicated that at full strength it was too concentrated for the mustard. In preparation for work with the sunflower on a different phase of mineral nutrition, therefore, it was decided to compare the effects on its growth of IR2S4 with dilutions of this solution and with solutions of other investigators (9, 18).

METHODS.—The plants were grown in the spring and summer of 1943 by essentially the same methods as previously used (3). The seeds were removed from the achenes, selected for uniformity, and planted—twelve per pot—in pure quartz sand in glazed 2-gallon pots, which were provided with a hole in the bottom for

drainage. Ten days to 2 weeks after planting, the plants were thinned to three uniform ones per pot. There were two crops; the growth period of the first was May 11 to June 17 (38 days); of the second, July 1 to August 5 (36 days). There were 359 plants in each crop, divided into five series of sixty plants and one series of fifty-nine. A different nutrient solution was used to water the plants of each of the six series.

Table 1 gives the molar concentrations of the salts of the six solutions which were compared, table 2 gives the parts per million of each element and the total for the salts, and table 3 gives the pH of the original solutions and of the drip obtained by flushing with distilled water or with the solutions.[1] The two flushings with nutrient solution were on different days. The pH was determined by means of a Coleman electrometer equipped with a glass electrode. The osmotic pressure of solution 3 (IR2S4) was 1 atmosphere (11), and the approximate osmotic pressure of solutions 1, 2, 4, 5, and 6 was 0.25, 0.5, 0.9, 0.7, and 0.5 atm., respectively. A solution of ferric citrate at a concentration of 1 p.p.m. of iron was sprinkled on the sand once a week, and copper chloride and zinc chloride at concentrations of 0.02 and 0.05 p.p.m., respectively, of copper and zinc were added every 2 weeks.

Enough was added to each pot daily until considerable solution drained out. The sand was flushed once a week, fresh nutrient solution being added immediately afterward.

Data

The weights of the plants produced by the six solutions are given in tables 4 and 5 and figure 1. In each crop the plants produced by solution 5 were the largest, followed in order by solutions 4, 3, 2, 6, and 1. The order is the same for each of the plant parts in the first crop, but in the second crop the roots of solution 5 weighed slightly less than those of solution 4, and the roots and leaves of solution 6 more than those of solution 2, although these differences are not great enough to be significant. Tables 4 and 5 also give the top-root ratios. These are consistently smaller for the second crop.

The data of tables 4 and 5 and figure 1 show that the plants grown with solution 3 rank third in size. In tables 6 and 7 the comparison is on a percentage ba-

TABLE 1

MOLAR CONCENTRATION OF SALTS OF NUTRIENT SOLUTION*

Solution	Salts				
	$Ca(NO_3)_2$	KH_2PO_4	$MgSO_4$	KNO_3	$(NH_4)_2SO_4$
1. IR2S4† (¼ conc.)	0.00225	0.00112	0.00112	....	....
2. IR2S4 (½ conc.)	0.00450	0.00225	0.00225	....	....
3. IR2S4 (full conc.)	0.00900	0.00450	0.00450	....	....
4. IR2S4 (low N)	0.00600	0.00450	0.00450	....	....
5. Hoagland and Arnon	0.00500	0.00100	0.00200	0.0050	....
6. Shive and Robbins	0.00450	0.00230	0.00230	....	0.00070

* Each solution also contained boric acid and manganese chloride at concentrations of 0.5 p.p.m. of each of the elements boron and manganese. A solution of ferric citrate at a concentration of 1 p.p.m. of iron was sprinkled on the sand once a week, and copper chloride and zinc chloride at concentrations of 0.02 and 0.05 p.p.m. of copper and zinc were added every 2 weeks.

† A solution of the LIVINGSTON triangle (11).

[1] Solutions are referred to hereafter mainly by number.

sis. The plants of solution 5 were more than 17% larger than those grown with solution 3, while those of solution 4 were more than 5% larger in the first crop and 9% in the second. The plants of the other solutions were smaller than those of solution 3—very much smaller in the case of solution 1.

about 6 miles from the greenhouse were obtained. While light and temperature (especially temperature) conditions at the airport and in the greenhouse would naturally differ, a comparison of the data for the two growth periods is not without significance in explaining the differences in the size of the plants of the two crops.

TABLE 2

ELEMENTS OF NUTRIENT SOLUTIONS (P.P.M.)

Solution	Ca	N	K	P	Mg	S	Mn	Bo	Total for salts
1. IR2S4 (¼ conc.)	90.2	63.0	44.0	34.9	27.4	36.1	0.5	0.5	661.7
2. IR2S4 (½ conc.)	180.4	126.1	88.0	69.7	54.7	72.1	0.5	0.5	1319.5
3. IR2S4 (full conc.)	360.7	252.1	175.9	139.4	109.4	144.3	0.5	0.5	2635.1
4. IR2S4 (low N)	240.5	168.1	175.9	139.4	109.4	144.3	0.5	0.5	2142.8
5. Hoagland and Arnon	200.4	210.1	234.6	31.0	48.6	64.1	0.5	0.5	1706.8
6. Shive and Robbins	180.4	145.7	89.9	71.3	55.9	96.1	0.5	0.5	1424.8

TABLE 3

pH OF ORIGINAL SOLUTIONS AND OF DRIP

Solution	Original solution	Drip by flushing with nutrient solution	Drip by flushing with nutrient solution	Drip by flushing with distilled water	Range of all determinations
1. IR2S4 (¼ conc.)	5.21	6.55–6.92	5.71–6.40	5.37–6.55	5.37–6.92
2. IR2S4 (½ conc.)	4.87	6.53–7.13	6.12–6.32	6.47–6.80	6.12–7.13
3. IR2S4 (full conc.)	4.61	5.57–6.17	5.67–6.30	5.55–5.80	5.55–6.30
4. IR2S4 (low N)	4.66	6.14–6.60	5.79–6.15	5.35–6.07	5.35–6.60
5. Hoagland and Arnon	5.00	6.81–7.82	6.73–7.56	6.71–7.92	6.71–7.92
6. Shive and Robbins	4.81	5.87–6.28	5.72–6.02	5.61–5.96	5.61–6.28

The data of tables 4 and 5 and figure 1 show that the plants of the second crop were considerably larger than those of the first. Table 8 places the comparison on a percentage basis. The effects of the higher temperatures and the greater amount of sunshine of the second growth period are clearly shown.

Continuous records of temperature and sunshine in the greenhouse where the plants were grown were not taken, but data of the U.S. Weather Bureau Station located at the municipal airport

The average daily maximum, minimum, and mean temperatures for the first growth period were 71.9, 54.5, and 63.2, respectively, and for the second 86.0, 65.6, and 75.8. There were only 2 days during the first period when the temperature reached 90° F. or above; there were 13 such days during the second. The sun shone 54.9% of possible shine during the first growth period and 68.8% during the second. These differences in temperature and sunshine are sufficient to account for the larger plants of the second crop.

In the case of each solution, the increased weights of the plants of the second crop were greatest for the roots and least for the leaves, the stems being intermediate. For the entire plant the increase ranged from 54% for solution 1 to 37% for solution 2, the other solutions being between these extremes.

But, as ARNON and HOAGLAND (1) and others have found, suitable and fairly uniform plant growth may be obtained with solutions varying considerably in both total concentration and salt proportions, provided there is present at each stage of growth a sufficient amount of each essential element. The present work supports this conclusion. While the plants grown with the various solutions varied considerably in size, their growth in all series was satisfactory, except those grown with solution 1 (tables 4, 5; fig. 1). This solution had an osmotic pressure of about 0.25 atm. and was evidently too dilute—at least under the conditions of this experiment—to supply sufficient nutrient elements. Tables 6 and 7 compare solution 3 (IR2S4) with the other solutions. The plants of solution 1 were 44.99% smaller than those of solution 3

TABLE 4

GRAMS PER 60 PLANTS, WET WEIGHT
(GROWTH PERIOD, MAY 11–JUNE 17)

Solution	Stems	Leaves	Tops	Roots	Entire plant	Top-root ratio
1. IR2S4 (¼ conc.)	1878.3	818.4	2696.7	364.0	3060.7	7.409
2. IR2S4 (½ conc.)	3220.2	1410.2	4630.4	482.5	5112.9	9.597
3. IR2S4 (full conc.)	3466.1	1529.2	4995.3	568.1	5563.4	8.793
4. IR2S4 (low N)	3659.0	1598.2	5257.2	614.6	5871.8	8.554
5. Hoagland and Arnon	4214.8	1677.0	5891.8	633.0	6524.8	9.308
6. Shive and Robbins	2981.4	1265.7	4247.1	394.3	4641.4	10.771

TABLE 5

GRAMS PER 60 PLANTS, WET WEIGHT
(GROWTH PERIOD, JULY 1–AUGUST 5)

Solution	Stems	Leaves	Tops	Roots	Entire plant	Top-root ratio
1. IR2S4 (¼ conc.)	2987.0	1019.5	4006.5	732.3	4738.8	5.471
2. IR2S4 (½ conc.)	4599.2	1498.5	6097.7	923.5	7021.2	6.603
3. IR2S4 (full conc.)	5174.0	1801.7	6975.7	1045.1	8020.8	6.675
4. IR2S4 (low N)	5727.0	1925.5	7652.5	1095.0	8747.5	6.989
5. Hoagland and Arnon	6389.4	2030.7	8420.1	1039.8	9459.9	8.098
6. Shive and Robbins	4353.0	1561.5	5914.5	924.1	6838.6	6.400

The top-root ratios of the second crop were smaller than those of the first (tables 4, 5). Table 8 shows that this is due to the relatively greater growth of the roots than of the tops during the second growth period.

Discussion

COMPARISON OF THE SIX SOLUTIONS

Much work has been done in an attempt to determine the best concentration and proportion of salts of the nutrient solution for the growth of plants.

in the first crop and 40.92% smaller in the second. The plants of the other four solutions were much closer to the size of the plants of solution 3, ranging from 17.94% larger in the case of solution 5 of the second crop to 16.57% smaller in solution 6 of the first. The plants of the other two solutions were between these extremes.

While satisfactory plants may be grown with solutions varying considerably in concentration and proportion of salts, this does not mean that consistent and significant differences may not be obtained by rather small variations in the nutrient solutions. As shown by figure 1, the plants of the second crop were considerably larger than those of the first, but the curves are of the same type. The difference between the plants of IR2S4 and each of the other solutions is the same in each crop within about 2–4% (tables 6, 7). Although the differences between IR2S4 and the other solutions are not large, except in the case of solution 1, they are probably significant. They are consistent in the two crops, and the number of plants of the two harvests of each series total 120 (119 in solutions 1 and 6).

But the results obtained with solutions varying in concentration or proportion of salts vary decidedly, depending on the method of application (**1**, **18**). If the solution is renewed intermittently or continuously at a low rate, or if the volume of solution is small, a higher concentration will be needed than with rapid continuous renewal. In the latter case the concentration may vary decidedly without materially affecting growth. By the continuous-renewal method, ROBBINS (**17**) secured excellent and uniform growth of tomato plants with solutions ranging in osmotic pressure from 0.44 to 1.70 atm. Even plants grown with a solution of 0.08 atm. approached in weight the plants of the solutions just mentioned, when the renewal was rapid. Continuous renewal also greatly diminishes changes in the pH and ionic proportions, owing to differential absorption of ions. In intermittent renewal these changes may be great enough decidedly to affect the growth of plants.

Different phyla of plants seem to vary as to the optimal concentration of the nutrient solution. VOTH (**21**) found that *Marchantia polymorpha* made the great-

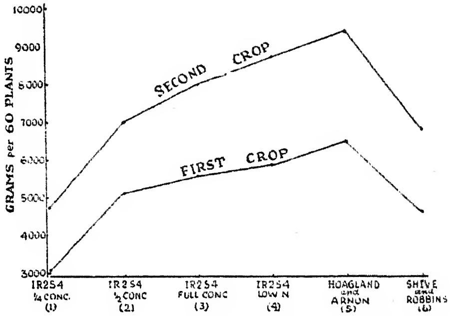

FIG. 1.—Comparison of weights of sunflower plants grown with six nutrient solutions. Growth period of first crop, May 11 to June 17; of the second, July 1 to August 5.

est vegetative growth on solutions ranging in osmotic concentration from 0.18 to 0.21 atm. In the present work the plants grown with solution 5 were the largest. This had an osmotic pressure of approximately 0.72 atm. In work with the mustard (**4**) it was found that when the solution was added daily to the sand, a nutrient solution of approximately 0.5 atm. resulted in larger plants than one of 1.0 atm. But there was no solution between 0.5 and 1.0 atm. It is possible that the plants would have been larger if a solution somewhat more concentrated than 0.5 atm. had been used. In both the mustard and the present work the nutrient solution was added to the sand once each day. It would seem that with

this type of application the optimal concentration for the mustard and sunflower lies between 0.5 and 1.0 atm. However, solution 5 also differs from the others in its salt proportions (table 1), and, as will be shown, this is another possible reason for its superiority.

Concentration alone can hardly account for the relative growth of the plants of solutions 1, 2, and 6 is probably due to the low concentration of their salts. Solutions 4 and 5, with approximate osmotic pressures of 0.90 and 0.72 atm. are probably about optimal in concentration. It would hardly be expected that solution 3 (IR2S4, osmotic pressure 1 atm.) would be too concentrated or that the slightly lower concentration of

TABLE 6

COMPARISON OF IR2S4 (NO. 3) WITH THE OTHER SOLUTIONS*

(GROWTH PERIOD, JULY 1–AUGUST 5)

Solution	Stems	Leaves	Tops	Roots	Entire plant
1. IR2S4 (¼ conc.)	45.81−	46.48−	46.02−	35.92−	44.99−
2. IR2S4 (½ conc.)	7.09−	7.78−	7.30−	15.07−	8.10−
4. IR2S4 (low N)	5.57+	4.51+	5.24+	8.19+	5.54+
5. Hoagland and Arnon	21.6 +	9.67+	17.95+	11.42+	17.28+
6. Shive and Robbins	13.98−	17.23−	14.98−	30.59−	16.57−

* Figures represent percentage increase or decrease in size of plants of other crops compared with IR2S4 and are obtained by dividing wet weights of plants of IR2S4 into the differences between these weights and those of corresponding plant parts of other solutions. Minus means smaller than IR2S4; plus means larger.

TABLE 7

COMPARISON OF IR2S4 (NO. 3) WITH THE OTHER SOLUTIONS*

(GROWTH PERIOD, JULY 1–AUGUST 5)

Solution	Stems	Leaves	Tops	Roots	Entire plant
1. IR2S4 (¼ conc.)	42.27−	43.41−	42.56−	29.93−	40.92−
2. IR2S4 (½ conc.)	11.11−	16.83−	12.59−	11.64−	12.46−
4. IR2S4 (low N)	10.69+	6.87+	9.70+	4.78+	9.06+
5. Hoagland and Arnon	23.49+	12.71+	20.71+	0.51−	17.94+
6. Shive and Robbins	15.87−	13.33−	15.21−	11.58−	14.74−

* See footnote to table 6.

plants in the various solutions. This is especially true of solution 5 (HOAGLAND and ARNON), as just mentioned. However, the other five solutions have the same salt proportions, except that solution 4 is somewhat lower in calcium nitrate and solution 6 contains a small amount of ammonium sulphate. The rather high magnesium and phosphate contents of solutions 3 and 4 may be important in accounting for the smaller plants of these solutions as compared with solution 5. The reduced size of the solution 4 (approximate osmotic pressure 0.9 atm.) would account for the larger plants of the latter solution in comparison with the former. Yet the only difference between these solutions is the somewhat lower calcium nitrate content of solution 4. This would be expected to lower—rather than to increase—growth. As shown by tables 6 and 7, the plants of solution 4 were 5% larger than those of solution 3 in the spring crop and 9% in the summer crop. The higher concentration of the latter may account for these

small differences. Concentrated solutions decrease water absorption and the plants assume a xerophytic aspect, as NIGHTINGALE (14), HAYWARD and LONG (7), and others have shown. Solution 3 was not concentrated enough to cause xerophytic characters, but it is possible that water absorption may have been lessened sufficiently to account for the slightly decreased growth of the plants of this in comparison with solution 4. In the previous work with the mustard (4), it was found that a solution of 1 atm. was too concentrated. As just given, the superiority of solution 4 is greater in the summer than in the spring, and this is to be expected if water absorption is the important factor.

POSSIBLE REASONS FOR THE SUPERIORITY OF SOLUTION 5

The plants of solution 5 were larger than those of any other solution. While the concentration of this solution is probably about optimal, it also differs from solutions 4 and 3 (ranking second and third, respectively, in salt proportions) and these differences may be important. (Solutions 1, 2, and 6 are not included in the comparison, for—as already mentioned—their low concentration probably accounts for the smaller plants of these solutions as compared with the other three.) Solution 5 is much lower in phosphorus, magnesium, and sulphur than are solutions 4 and 3 (table 2). While the low sulphur content is probably not significant, the small amount of magnesium and phosphorus may be. Also, in contact with the roots of the plant, solution 5 attains a much higher pH than is true of the other solutions. However, this high pH would be expected to inhibit rather than to increase the growth of the plants.

TABLE 8

COMPARISON OF FIRST AND SECOND CROPS*

Solution	Stems	Leaves	Tops	Roots	Entire plant
1. IR2S4 (¼ conc.)	59.03	24.57	48.57	101.18	54.83
2. IR2S4 (½ conc.)	42.82	6.26	31.69	91.40	37.32
3. IR2S4 (full conc.)	49.27	17.82	39.65	83.96	44.17
4. IR2S4 (low N)	56.52	20.48	45.56	78.16	48.97
5. Hoagland and Arnon	51.59	21.09	42.91	64.27	44.98
6. Shive and Robbins	46.01	23.37	39.26	134.36	47.34

* Figures represent percentage increase in weights of plants of second crop compared with first and are obtained by dividing wet weights of plants of first crop into the differences between these weights and those of corresponding plant parts of second crop.

LOW PHOSPHATE CONTENT

There is considerable work indicating that a plant growing in either soil or a nutrient solution can obtain all the phosphorus it can use from solutions of very low phosphate content. In solution cultures, TEAKLE (19) found that 1 p.p.m. of phosphate produced practically as large plants as larger amounts. HOAGLAND and MARTIN (8) found 9 p.p.m. of phosphate optimal in one experiment, but in the second experiment the plants grown with 1.1 p.p.m. were almost as large as when higher concentrations were used. Using large volumes of culture solution and renewing the phosphate content once or twice a day, PARKER (15) secured maximum growth of corn and soybean with 0.5 p.p.m. of phosphate. The results indicated that if the phosphate content could have been

maintained constant, a still lower concentration would have sufficed for maximum growth. In a companion piece of work, PIERRE and PARKER (16) found that the inorganic phosphate content of the displaced solution of several soils giving no response to phosphorus fertilization was only 0.03–0.04 p.p.m. (The organic phosphorus of the displaced solution was unavailable to plants.) This is considerably lower than that found optimal by PARKER for growth of corn and soybean and indicates that the displaced solution does not represent completely the solution from which the phosphorus is absorbed.

Since maximum growth of plants may occur with nutrient solutions containing phosphorus in very low concentrations, it would be expected that care should be used to avoid toxic effects due to a too high concentration of this element. MOORE (12) found that when the peanut was grown in the light, phosphorus at a concentration ordinarily used in nutrient solutions was toxic but not when the plant was grown in the dark. However, the nitrate ion seems to antagonize, to a degree at least, the toxic effects of the phosphate ion. VOTH and K. C. HAMNER (22) reported that greater growth of *Marchantia polymorpha* occurred in those solutions low in phosphate and high in nitrate. C. L. HAMNER (6) found that if nitrogen was deficient, phosphorus in low concentrations was toxic to the soybean, but that this toxicity disappeared with greater amounts of nitrogen. In general, at least in the younger stages of growth, the toxic effect of two parts of phosphate was overcome by one part of nitrate. BECKENBACH, WADLEIGH, and SHIVE (2) varied the concentrations of both the cations and anions of the nutrient solution. In the case of the anions the solution high in nitrate and low in phosphate and sulphate gave the greatest growth. This is attributed mainly to the effect of the nitrate. They do not discuss the nitrate-phosphate relationship.

As shown by table 2, the nitrogen-phosphorus ratio of solution 5, the plants of which were the largest, is much greater than those of solutions 4 and 3, which ranked second and third. So the relatively low phosphorus content in proportion to the nitrogen may be one reason for the superiority of solution 5. Even this solution is high in phosphorus compared with the concentrations reported optimal by the investigators just mentioned. Solution 5 contains 31 p.p.m. of phosphorus (95 p.p.m. of phosphate). Perhaps the plants would have been larger if the concentration of phosphorus had been even lower.

LOW MAGNESIUM CONTENT

Another factor possibly contributing to the superiority of solution 5 is the high Ca/Mg ratio of this solution in comparison with solutions 4 and 3. As shown by table 2, solution 5 contains less than half as much magnesium as the other solutions. The Ca/Mg ratios of solutions 5, 4, and 3 are, respectively, 4.12, 2.19, and 3.29.

The toxic effects of magnesium and the reduction of the toxicity by calcium are well known. While some have concluded that there must be a rather definite Ca/Mg ratio for satisfactory growth of plants, much of the literature indicates that the ratio may vary widely without injurious effects. LIPMAN (10) gives a comprehensive review of field experiments on the lime/magnesia ratio in soils. He finds little or no support for the idea that it is necessary to have in soils a proper lime/magnesia ratio. Much of the more recent work with solution and sand cultures is to the same effect. BECK-

ENBACH *et al.* (2) found that the Ca/Mg ratio was not significant in the growth of corn, and GAUCH (5) found no well-defined Ca/Mg ratio which was optimal for the growth of bean.

While it may be hard to demonstrate the necessity of a definite Ca/Mg ratio for the growth of plants, this does not mean that the amount of magnesium in the nutrient solution is not important. High concentrations of magnesium result in smaller plants. It is possible that growth of the plants of solutions 4 and 3 was reduced owing to the presence of too much magnesium and that the low magnesium content of solution 5 in proportion to the calcium is one reason for the greater growth of the sunflower in this solution. But this should not be overemphasized. It would seem that solutions 4 and 3 are sufficiently high in calcium to prevent inhibition of growth by the magnesium. However, the antagonism might be incomplete.

HIGH PH OF DRIP FROM THE POTS

As shown by table 3, the pH of the drip of solution 5 ranged 6.71–7.92, higher than any other solution. Three-fourths of the determinations were alkaline. The nitrate content of solution 4 approaches—and of solution 3 exceeds—that of solution 5 (table 2). The first two solutions are much higher in potassium phosphate than solution 5, with the result that the pH of their drip is lower. Before application to the sand, the pH of the solutions did not vary widely.

The greater absorption of cations than anions causes a decrease in pH. Solution 6 contained a small amount of ammonium sulphate, and the pH of its drip does not rise as high and shows less fluctuation than any other solution. TRELEASE and TRELEASE (20) found that with a certain nitrate/ammonium ratio there was very little change in the pH of the nutrient solution.

Even with the high pH of solution 5, growth of the sunflower was not inhibited. The plants were the largest of all the series. They might have been still larger at a lower pH, but their appearance did not indicate any adverse effects of the high pH—such as the deficiency of one or more of the manganese, iron, and phosphate ions. Iron was added as ferric citrate. If other ions, such as manganese or phosphate, were precipitated, it would only be after they were applied to the sand. The original solution was acid enough to keep them in solution. If precipitation occurred in the sand, it would probably be a finely divided one, which on accumulation might dissolve sufficiently to give the plants an adequate supply of the ions.

Summary

1. By means of sand cultures, solution 3 (IR2S4 of the LIVINGSTON triangle, osmotic pressure 1.0 atmosphere) was compared in the growth of the sunflower with dilutions of this solution and with solutions of other investigators. Six solutions were compared.

2. The plants grown with solution 5 (HOAGLAND and ARNON) were the largest of any series, 17% larger than those of solution IR2S4. Its superiority was probably due to its nearly optimal concentration (approximate osmotic pressure 0.7 atm.), its low phosphate content in proportion to nitrate, and its low magnesium concentration in relation to calcium.

3. The plants of solutions 1, 2, and 6 (approximate osmotic pressure 0.25, 0.50, and 0.50 atm., respectively) were smaller than those of IR2S4. Expressed in percentages, the range was 44.92% smaller in the case of solution 1 of the

first crop to 8.10% in solution 2 of the same crop, the other differences being between these extremes. These three solutions were probably too dilute for best growth of the sunflower.

4. Solution IR2S4 with an osmotic pressure of 1 atm. seemed too concentrated. The composition of solution 4 was the same as IR2S4, except that the former solution was somewhat lower in calcium nitrate. This would be expected to decrease rather than to increase growth. Yet, probably because of a more optimal concentration, the plants of solution 4 (approximate osmotic pressure 0.9 atm.) were larger by 5.0–9.0% than those of IR2S4.

5. Both solutions 3 (IR2S4) and 4 produced plants inferior to those of 5. Their inferiority was probably due to their higher concentrations and higher contents of phosphate and magnesium.

6. The pH of the drip of solution 5 was higher than that of any other solution, most of the determinations showing an alkaline reaction. This high pH was due to the facts that all the nitrogen was supplied as nitrate and the content of potassium phosphate was quite low. Solution 6 (SHIVE and ROBBINS) contained a little ammonium sulphate, and the pH of its drip did not rise so high and showed less fluctuation than any other solution.

7. Because of higher temperatures and more sunshine, the plants of the second harvest were considerably larger than those of the first. The increased growth of the former plants in comparison with the latter was greater for the roots than for the tops, so that the top-root ratios of the second crop were smaller.

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LITERATURE CITED

1. ARNON, D. I., and HOAGLAND, D. R., Crop production in artificial culture solutions and in soils with special reference to factors influencing yields and absorption of inorganic nutrients. Soil Sci. **50**:463–484. 1940.
2. BECKENBACH, J. R., WADLEIGH, C. H., and SHIVE, J. W., Nutrition studies with corn: I. A statistical interpretation of the nutrient ion effect upon growth in artificial culture. Soil Sci. **41**:469–486. 1936.
3. EATON, S. V., Influence of sulphur deficiency on metabolism of the sunflower. BOT. GAZ. **102**: 536–556. 1941.
4. ———, Influence of sulphur deficiency on metabolism of black mustard. BOT. GAZ. **104**:306–315. 1942.
5. GAUCH, HUGH, Responses of the bean plant to calcium deficiency. Plant Physiol. **15**:1–21. 1940.
6. HAMNER, C. L., Growth responses of Biloxi soybeans to variation in relative concentrations of phosphate and nitrate in the nutrient solution. BOT. GAZ. **101**:637–649. 1940.
7. HAYWARD, H. E., and LONG, E. M., Anatomical and physiological responses of the tomato to varying concentrations of sodium chloride, sodium sulphate, and nutrient solutions. BOT. GAZ. **102**:437–462. 1941.
8. HOAGLAND, D. R., and MARTIN, J. C., A comparison of sand and solution cultures with soils as media for plant growth. Soil Sci. **16**:367–388. 1923.
9. HOAGLAND, D. R., and ARNON, D. I., The water culture method for growing plants without soil. California Agr. Exp. Sta. Circ. 347. 1938.
10. LIPMAN, C. B., A critique of the hypothesis of the lime/magnesia ratio. Plant World **19**:83–105;119–135. 1916.
11. LIVINGSTON, B. E. (Editor), A plan for co-operative research on the salt requirements of representative agricultural plants. 2d ed. Baltimore. 1919.
12. MOORE, R. H., Nutritional levels in the peanut plant. BOT. GAZ. **98**:464–490. 1937.
13. NIGHTINGALE, G. T., and BLAKE, M. A., Effects of temperature on the growth and metabolism of Elberta peach trees with notes on the growth responses of other varieties. New Jersey Agr. Exp. Sta. Bull. 567. 1934.
14. NIGHTINGALE, G. T., and FARNHAM, R. B., Effects of nutrient concentration on anatomy, metabolism, and bud abscission of sweet pea. BOT. GAZ. **97**:477–517. 1936.
15. PARKER, F. W., Soil phosphorus studies: III. Plant growth and the absorption of phosphorus

from culture solutions of different phosphate concentrations. Soil Sci. 24:129–146. 1927.

16. PIERRE, W. H., and PARKER, F. W., Soil phosphorus studies: II. The concentration of organic and inorganic phosphorus in the soil solution and soil extracts and the availability of the organic phosphorus to plants. Soil Sci. 24:119–128. 1924.
17. ROBBINS, W. R., Relation of nutrient salt concentrations to growth of the tomato and to the incidence of blossom-end rot of the fruit. Plant Physiol. 12:21–50. 1937.
18. SHIVE, J. W., and ROBBINS, W. R., Methods of growing plants in solution and sand cultures. New Jersey Agr. Exp. Sta. Bull. 636. 1938.
19. TEAKLE, L. J. H., The absorption of phosphate from soil and solution cultures. Plant Physiol. 4:213–232. 1929.
20. TRELEASE, S. F., and TRELEASE, HELEN M., Changes in hydrogen-ion concentration of culture solutions containing nitrate and ammonium nitrogen. Amer. Jour. Bot. 22:520–542. 1935.
21. VOTH, P. D., Effects of nutrient-solution concentration on the growth of *Marchantia polymorpha*. BOT. GAZ. 104:591–601. 1943.
22. VOTH, P. D., and HAMNER, K. C., Responses of *Marchantia polymorpha* to nutrient supply and photoperiod. BOT. GAZ. 102:169–205. 1940.

THERMOREGULATION IN THE EXPERIMENTAL GREENHOUSE

K. STARR CHESTER AND W. WINFIELD RAY

It has been shown by a number of investigators (DODOV, GASSNER and STRAIB, RUDORF, ROBERTS, GESHELE, HASSEBRAUK, NEWTON and JOHNSON, and CHESTER) that the reactions of wheat varieties used in differentiating physiological races of wheat leaf-rust (*Puccinia triticina* Eriks.) may differ so markedly in response to greenhouse temperatures that race identifications by different workers—or by the same worker at different times—are often not comparable. The equipment described here was installed to overcome this and other similar problems in pathological research.

The marked advances in methods for uniformly heating buildings during the past few years offer the possibility of applying the same principles to the experimental greenhouse. In 1943, thermostatically controlled projection steam heaters, such as are used for thermoregulation of institutions and commercial buildings, were installed in two of the experimental greenhouses of the Oklahoma Agricultural Experiment Station. The results were so satisfactory that the essential features of the installations are briefly presented here. The writers will be glad to furnish additional information on details of the installation, operation, and the relatively low cost of the equipment ($200 per house under existing conditions).

The two greenhouses selected were nearly square, with 800 sq. ft. of floor space, height of 15 ft. at the center, and volume of 7000 cu. ft. It was desired to maintain the temperatures of the two houses as constantly as possible at 65° and 80° F., respectively. In each greenhouse, at the center and 13 ft. from the floor, was installed a projection unit heater consisting of a hollow coil of small steam tubes surrounding an electric fan (1150 r.p.m.) which projected the heated air to all parts of the greenhouse by directing it at an anemostat or diffuser having four cone-shaped baffles (fig. 1). At a convenient point midway between heater and outer walls was located a pair of mercoid-type line voltage thermostats, one of which activated the heater fan, the other an alarm bell located at a distant point in the greenhouse system where an attendant was on duty throughout the day. There was no at-

tendant on duty at night, nor was one needed. The steam supply to the standard greenhouse system of heating pipes (which were undisturbed) was reduced to provide the greenhouse with only sufficient heat to warm it to a point approximately 15° F. lower than the temperature desired, and the projection

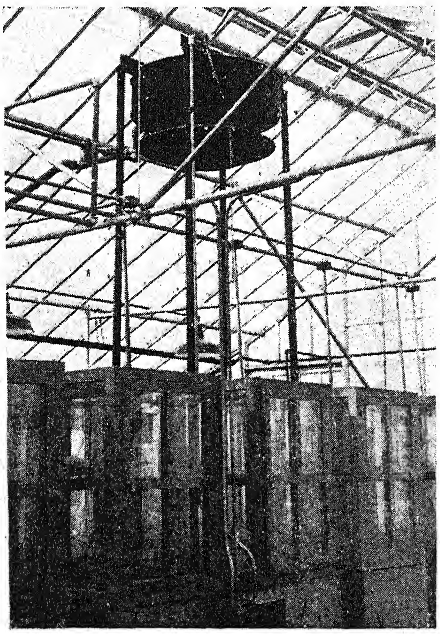

FIG. 1.—Projection steam heater and diffuser installed for thermoregulation of greenhouse. Battery of constant soil-air temperature cabinets shown below.

heater provided the remaining heat required.

The installation was semi-automatic to this extent: the thermostat activated the heater within a 1° limit if the temperature dropped. If, because of the sun's radiation or rising outdoor temperatures, the temperature rose as much as 3° above that desired, the second thermostat activated the alarm bell as a signal for the attendant to open the ventilators. Usually the alarm bell rang once a day, about 2 hours after sunrise, and this was soon forestalled by more prompt opening of the ventilators.

With lack of means for cooling the greenhouses, the equipment is useful only during the period in which outdoor temperatures are 10° or more lower than the desired greenhouse temperatures (approximately October–March for the 65° greenhouse and September–May for the 80° greenhouse under Oklahoma conditions); nevertheless these periods are adequate for an extensive program of greenhouse research during the period in which field experiments are inactive.

The efficiency of the equipment is indicated by the typical thermograph records in figure 2. They show temperatures in the "65°" greenhouse for January 5–12, 1942 and January 10–17, 1944, before and after the thermoregulatory equipment was installed. That outdoor temperatures were similar for the 2 weeks is shown by the dotted lines connecting outdoor maximum and minimum daily temperatures at a point a few rods from the greenhouse during the same periods. With thermostatic regulation, the temperatures for the week did not once depart from the range 62°–68° F., while in the same greenhouse in a comparable week with manual control the temperatures were within this same range only 38% of the time. The slight variations seen in the lower record are principally the result of temperature fluctuations due to the opening of doors and other disturbances in connection with watering and care of the greenhouse.

The efficiency of this means of thermoregulation is also reflected in the results of infection experiments and in the normalcy of plant growth. Prior to installation of the equipment it was not unusual to have 50% of infection tests with wheat leaf-rust result in failure because

of excessively high or low temperatures, particularly in the 6–8 hour period following inoculation. Since the equipment was installed there has not been a single failure from this cause. With such temperature control and 50 candle-power of supplementary light in the 65° greenhouse at night, winter wheat has reached heading stage in slightly more than 2 months after planting, which indicates the possibility of raising two generations of wheat per year in breeding experiments, even during the relatively brief fall-to-spring season in the latitude of Oklahoma.

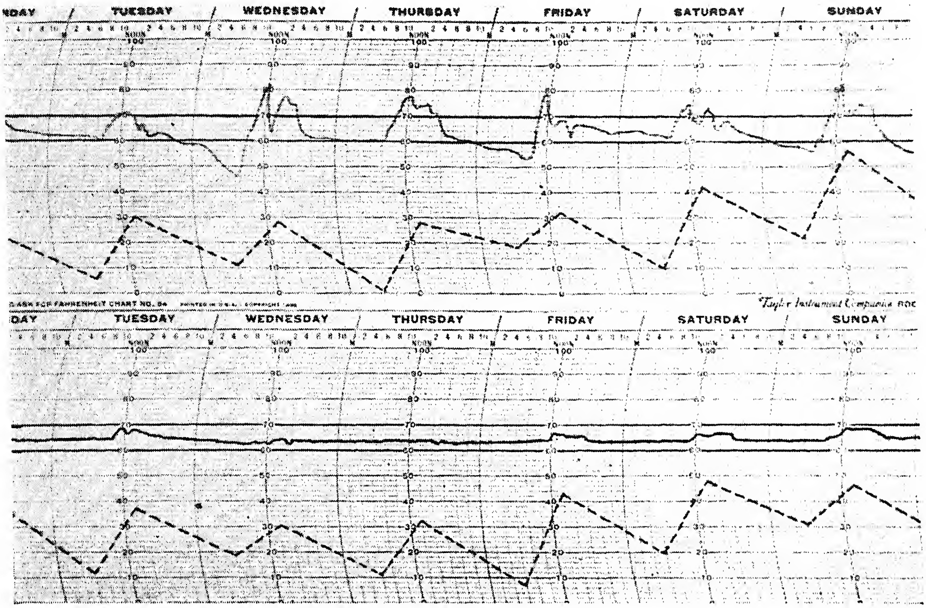

FIG. 2.—Record of attempts to maintain uniform temperature of 65° in greenhouse. Upper, before installation of thermoregulatory equipment (Jan. 5–12, 1942); lower, after installation (Jan. 10–17, 1944). Solid lines show greenhouse temperatures; dotted lines connect daily outdoor maxima and minima.

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A CHAMBER FOR GROWING PLANTS UNDER CONTROLLED CONDITIONS

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A chamber has been devised, illuminated by fluorescent lamps, in which several species of plants have been grown from seed to maturity. In these chambers relatively accurate control is exercised over most of the environmental factors which influence plant growth and development. Other investigators (1, 2) have found the fluorescent type of lamp satis-

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factory as the sole source of illumination for growing plants, in that they are more efficient than tungsten filament types and produce less heat for a given amount

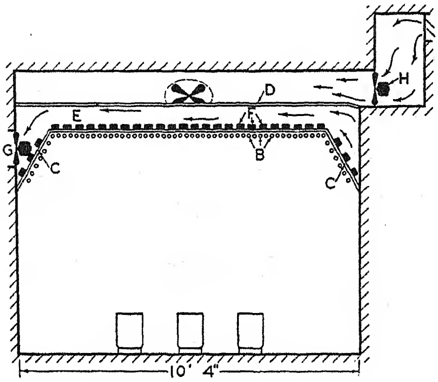

FIG. 1.—Diagrammatic cross-section of control chamber, about 4 feet from end of room near which lighting equipment is placed. The fan (center), which serves to recirculate the air in the room, is shown encircled with broken lines, since it would not fall in the plane of this section. Three pots shown in position.

of illumination. Almost any desired quality may be obtained, but intensities above 2000 foot-candles are impossible to secure.

Description

Figures 1 and 2 show end and side elevations of the chamber and the equipment installed in it. The chamber is a basement room approximately $10\frac{1}{2} \times 16\frac{1}{2}$ feet, with three interior walls of cinder block and an outside (foundation) wall of concrete. Three 2×6 joists were installed across the room, with clearance of $6\frac{1}{2}$ feet from the floor (fig. 2*A*). To these were attached eight light panels of wood, 2×3 feet, each carrying twelve 30-watt, 36-inch fluorescent tubes on the lower side and the accessory equipment for the tubes above. The lower surfaces of the panels were painted white, as reflectors, and the upper surfaces were covered with heavy asbestos as a protection between the wood and the accessory equipment. This installation formed a false ceiling 6×8 feet near one end of the room and equidistant from the side walls. Four similar panels, each carrying six fluorescent tubes, were installed between the edge of this false ceiling and the side walls of the room at an angle of about 60° (fig. 2*C*) with the wall; and finally, two 6-tube panels were suspended at

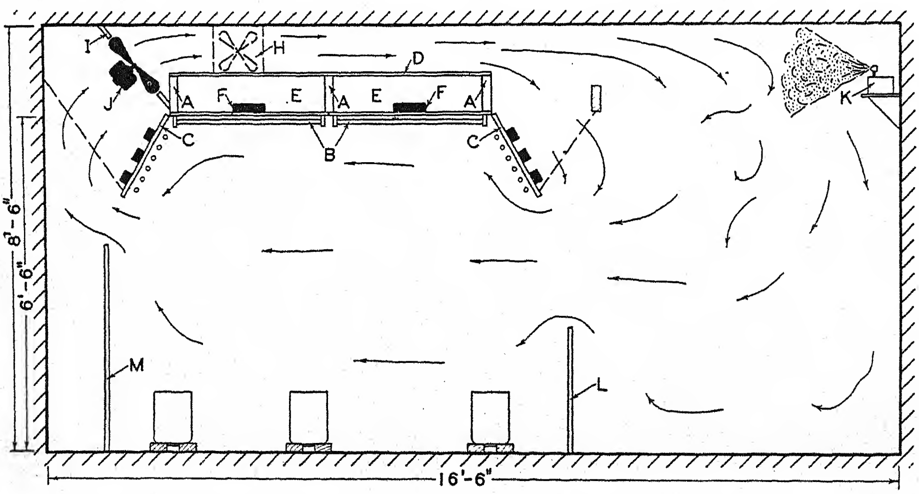

FIG. 2.—Diagrammatic longisection of control chamber. Fan *H*, which controls entrance of outside air, shown in broken lines to indicate its relative position. Three pots shown in position.

each of the other two edges of the false ceiling, at an angle of about 45° with the vertical. Thus, the light source consisted of 144 30-watt fluorescent tubes.

Plywood (fig. 2*D*) across the top of the joists holding the lamp panels formed two horizontal ducts (*E*) between the joists, the floor of the ducts consisting of the lamp panels, and with the accessory equipment for the tubes attached to this floor. The ducts were extended to the side walls of the room by the use of plyboard, and openings were provided at the ends of the ducts so that air from the chamber could be drawn through them and exhausted from the room through a hole in the side wall by a fan (fig. 1*G*), thus removing most of the heat generated by the accessory equipment of 122 of the lamps. The exhaust fan ran all the time the lights were on.

Just above the ducts, and at the wall opposite the exhaust fan, an intake fan (fig. 1*H*) was installed at an opening to the outside of the building. It was thermostatically controlled to draw in cool outside air whenever the room temperature rose to a given point.

A plywood sheet (fig. 2*I*) was placed at an angle of about 45° from the horizontal lamp panels to the ceiling of the chamber, blocking communication between air above and below the lamp and duct assembly at the end of the chamber. A 14-inch fan (*J*) was installed in this barrier and operated continually to circulate the air in the chamber.

A white-painted baffle (fig. 2*L*) 2 feet high was set on the floor, across the chamber opposite the circulating fan *J* and just beyond the location of the lamp panels. A second baffle (*M*) was installed similarly beneath the fan. These served as reflectors and produced eddy currents that insured thorough air circulation throughout the chamber. Near the ceiling at the end of the room opposite the lamp arrangement was installed a Spray-

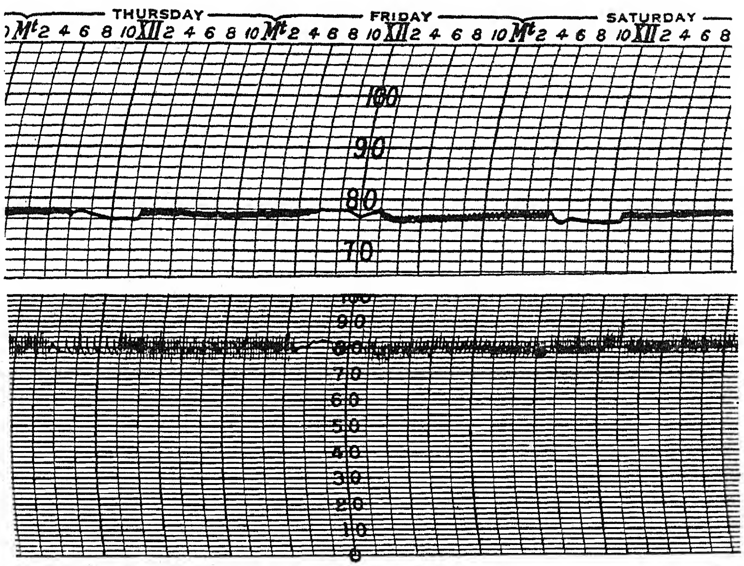

FIG. 3.—Portion of typical weekly hygrothermograph page. Above, temperature graph; below, graph of percentage relative humidity.

co[2] humidifier (*K*) with three nozzles activated by compressed air. When actuated by a horse-hair hygrostat located near the recirculating fan *J*, this humidifier delivered moisture as a fine fog that evaporated almost at once.

Discussion

In preliminary work, many types of fluorescent tubes were tried to determine what quality of light was most satisfactory, and it was found that the best growth of the plants tried was obtained through the use of white fluorescent tubes alternating with the daylight tubes.

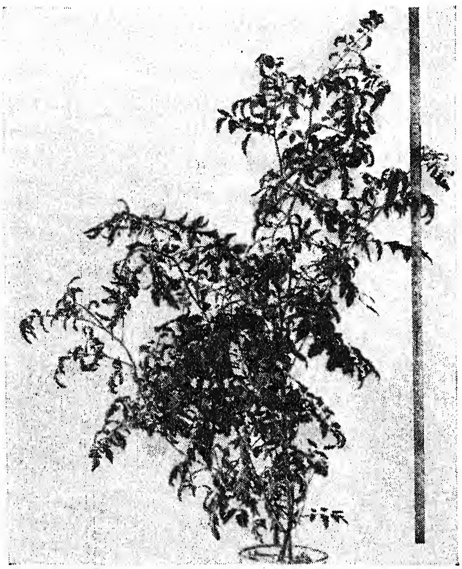

FIG. 4.—Tomato plant 11 weeks old, grown from seed in control chamber continuously. Some of the fruit from this plant had been harvested for analytical purposes.

Three chambers of this kind have been in actual operation for more than a year, with satisfactory results. Figure 3 shows a portion of a weekly hygrothermograph record from one of the rooms. In this particular case the lights burned for approximately 15 hours each day, and in the remaining 9 hours the rooms were in darkness. It has been our experience that more nearly uniform records are obtained when the chambers are illuminated continuously. During the coldest months of the year it has been necessary to supply supplementary heat during the hours of darkness if the temperature of the chamber is to be maintained fairly high. This may be accomplished through the use of small electric heaters or any other heating system which will operate continuously during the dark period.

Plants of Biloxi soybean, red kidney bean, and tomatoes have been grown successfully from seed to maturity, and many other plants have been grown satisfactorily during experiments which did not involve seed production. Figure 4 shows a tomato plant which was grown in the room for 11 weeks at a temperature of $77° \pm 1°$ F. and a relative humidity of $80 \pm 5\%$. Many of the fruits had been removed from this plant for analytical purposes. A fairly heavy crop was produced and ripened satisfactorily. Practically all the leaves were still present, and nearly all were of a uniform deep-green color. In appearance these plants were comparable with those of the same age which had been grown in the greenhouse during the late spring. Plants grown in these chambers from November to April have always produced nearly twice as much vegetation as those in the greenhouse at Ithaca, New York. Outdoors during the summer months, tomato plants produced about 30% more dry weight and about 50% more fruit than did those in the control room. The temperature during the dark period was fairly high in the control chamber, however, and it might have been possible to grow

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plants more nearly comparable with those outdoors if other temperatures had prevailed. This would have been true more particularly if the temperature during the dark periods had been lowered.

LITERATURE CITED

1. NAYLOR, A. W., The use of fluorescent light in experimental work. Trans. Illinois State Acad. Sci. 34:82–84. 1941.

2. NAYLOR, A. W., and GERNER, GLADYS, Fluorescent lamps as a source of light for growing plants. BOT. GAZ. 101:715–716. 1940.

ANATOMICAL RESPONSES OF TOMATO STEMS TO VARIATIONS IN THE MACRONUTRIENT CATION SUPPLY

C. B. LYON[1] AND C. R. GARCIA[2]

Introduction

In a previous study (6), variations in nitrate, sulphate, and phosphate supplied to tomato plants grown in sand culture resulted in differences in the anatomy of tomato stems. Cellular differences which could be associated with nutrient supply were described. Differences in the actual—as well as the relative—area of component tissue systems in stem cross-sections were reported, and these characteristics were correlated with growth and fruitfulness of the plants.

It is the purpose of this paper to complete the survey of anatomical differences in tomato stems which result from variations in the relative proportions of macronutrient ions supplied and to correlate observed differences with the vegetative and fruitful condition of the plant. The results with respect to variations in the calcium, potassium, and magnesium supply are reported.

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Material and methods

The material was obtained from the 1941 experiment of HAMNER, LYON, and HAMNER, described in detail elsewhere (4). Briefly, an inbred strain of Bonny Best variety of tomatoes was planted in the greenhouse May 12, 1941, and the seedlings were transplanted to 2-gallon glazed crocks containing pure quartz sand. On July 1, all plants were placed outdoors. The plants were harvested September 10.

The design of the experiment was that of a randomized block (2) with eighty-seven treatments and four replications. Each replication consisted of a three-plant row, and the replications were randomized by the use of TIPPETT's randomization tables (8). Thus, the mean of twelve plants is used as an estimation of the results produced by a given treatment. The data were reduced by means of the analysis of variance, and the t test (7) was used for determining whether particular differences were significant. Odds as great as or greater than 99:1 were accepted as statistically significant that the observed

deviations were not due to errors of random sampling. Subsequent to the time the seedlings were transplanted, the same randomization and design was maintained, both in the greenhouse and in the field.

In the forty-three solutions considered in this investigation, the relative proportions of calcium, potassium, and magnesium were varied, while the total equivalent concentration of these ions was constant. Each solution contained 12.0 milliequivalents per liter of nitrate, 4.5 me./l. of phosphate, and 9.0 me./l. of sulphate. For convenience, the forty-three solutions are represented in a cation triangle (fig. 1), and the treatment numbers are assigned to those solutions used. All solutions contained equal amounts of the micronutrients in the following concentrations:

B (as H_3BO_3)	0.5 p.p.m.
Mn (as $MnCl_2 \cdot 4H_2O$)	0.5
Zn (as $ZnSO_4 \cdot 7H_2O$)	0.05
Cu (as $CuSO_4 \cdot 5H_2O$)	0.02
Fe (as ferric citrate)	5.0

When the plants were 120 days old and had received their respective nutrient treatments for 94 days they were harvested, and a section of the stem from the middle internode of each plant was preserved for anatomical study. The stem sections were fixed with Navashin's solution, and air was evacuated from the tissues. The material was dehydrated in an ethyl-tertiary butyl-alcohol series and infiltrated with a paraffin-beeswax rubber mixture. Complete cross-sections were cut at 10–20 μ and stained with a modified Flemming's triple stain. The material was mounted in balsam.

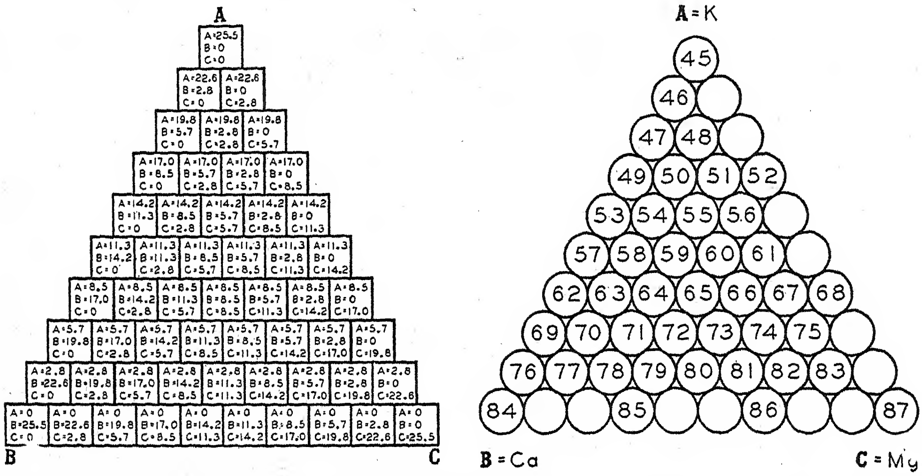

FIG. 1.—Left, macronutrient cation composition (me. /l.) of nutrient solution for 55 possible treatments. Right, treatments used, with treatment numbers assigned.

All stem sections were projected at a constant magnification of approximately 30 diameters. The images were centered on a screen marked off with four diameters dividing the stem section into eight equal sectors, as previously described (6). The radial measurements of each tissue system on each of the eight radii were recorded. Thus, the mean of eight measurements was used for xylem, phloem, and cortex; the mean of four measurements was used for pith and stem diameter.

In these measurements the internal

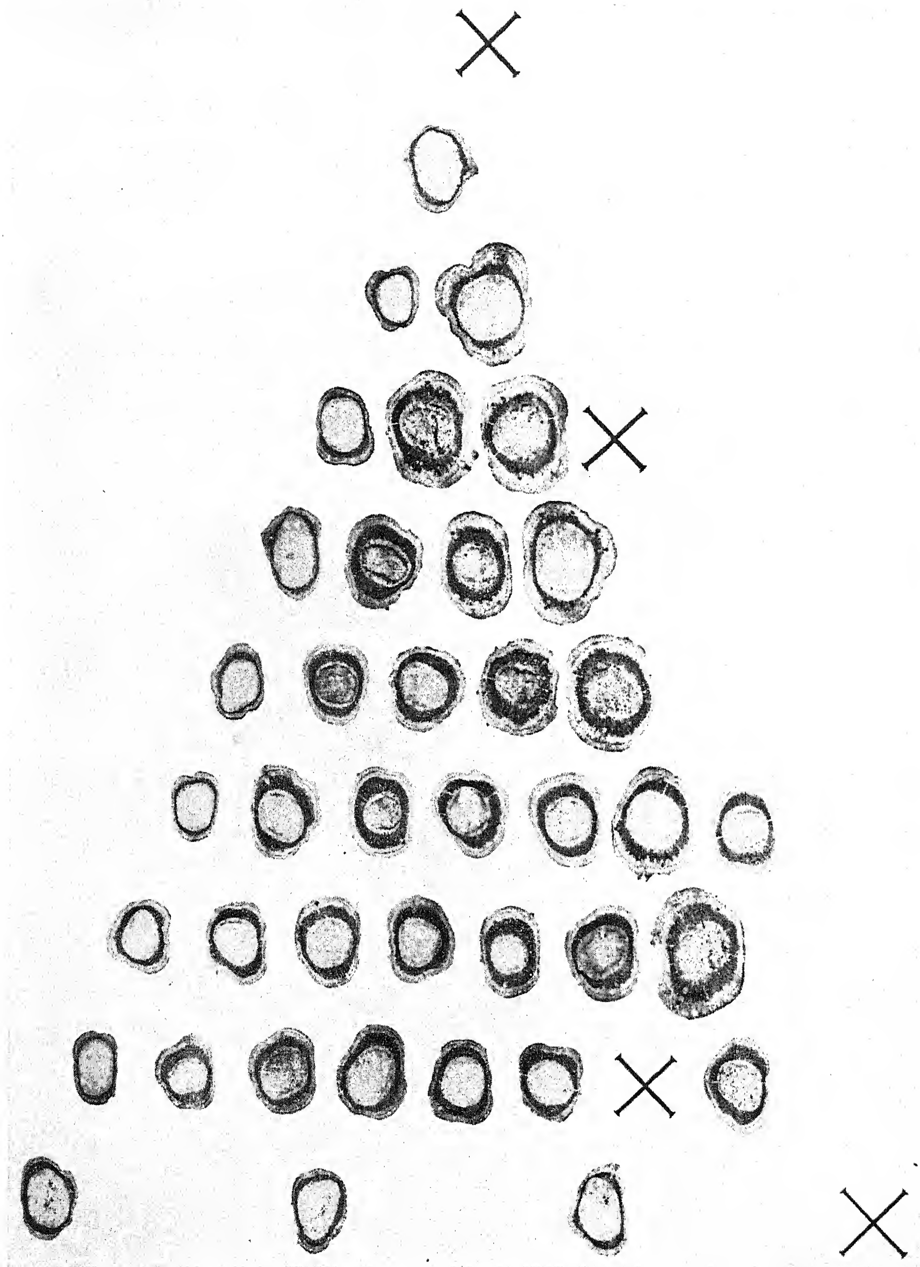

FIG. 2.—Typical cross-sections of tomato stems from plants in cation treatments (corresponding to those shown in fig. 1).

phloem was disregarded, and the term "pith" includes all cells internal to primary xylem; "xylem" includes all cells from primary xylem to cambium; "phloem" includes all cells from cambium to pericycle; "cortex" includes all cells from endodermis to and including cuticle.

The mean radial measurement of any tissue system for a given stem section was used to calculate the actual area of that system in the cross-section. Standard formulas developed for circles were employed. It was considered that the variability within a stem attributable to the deviations of the tissue systems from circles was nullified by the use of mean values. Thus, all inter-treatment comparisons consider only differences between stems with respect to areas of the tissue systems. Two types of such comparisons were made for each tissue system: (*a*) actual areas were computed and treatment means were compared, and (*b*) the percentage cross-sectional area of the stem section occupied by the area of any given tissue system was computed and treatment means compared. In this way the actual size as well as the relative amount of tissue in a stem section was evaluated.

Results

Photomicrographs of cross-sections from typical tomato stems in each treatment are given in figure 2. Large differences—which include stem size, tissue system development, starch accumulation, and to some extent cellular anatomy—are demonstrable. These characteristics will be discussed individually.

This experiment had been specifically designed so that data obtained for any character could be analyzed for the presence or absence of statistically significant differences. Accordingly, analyses of variance were computed for each individual character, and the results are compiled in table 1. Plants from several treatments supplied with nutrient solution lacking calcium were discarded owing to the complete deterioration of terminal meristems, and several plants were discarded as a result of mechanical

TABLE 1

F VALUES FROM ANALYSES OF VARIANCE FOR ANATOMICAL CHARACTERS OF TOMATO STEM CROSS-SECTIONS

Anatomical characters	Source of variation		
	Between treatments	Between blocks	Within blocks
Percentage			
Cortex	12.67*	0.86	0.77
Phloem	25.74*	0.31	1.51
Xylem	18.40*	0.80	4.98*
Pith	22.64*	0.64	5.84*
Area			
Cortex	20.97*	2.23	0.01
Phloem	34.24*	2.48	0.52
Xylem	24.10*	2.52	0.93
Pith	6.19*	1.55	1.53
Stem diameter	20.07*	2.42	0.24

* Highly significant, satisfying statistical requirements for P = 0.01.

injury. This resulted in data which were non-orthogonal. The sources of variation are:

Variation due to	No. of degrees of freedom
Treatments	38
Between blocks	3
Within blocks	2
Error	374
Total	417

The substitution of missing values by Yates' method (9) did not significantly change the results of analyses (table 1), and they are considered valid.

It is evident that treatments produced statistically significant differences in all the characters listed. It is also evident

that differences in environment, when measured by replications within blocks, produced significant differences in percentage cross-sectional area occupied by xylem and pith. Since replication differences were measurable in the analysis of variance, and since these differences were not included in inter-treatment comparisons, valid differences exist in all the listed characters which can be ascribed directly to the treatments.

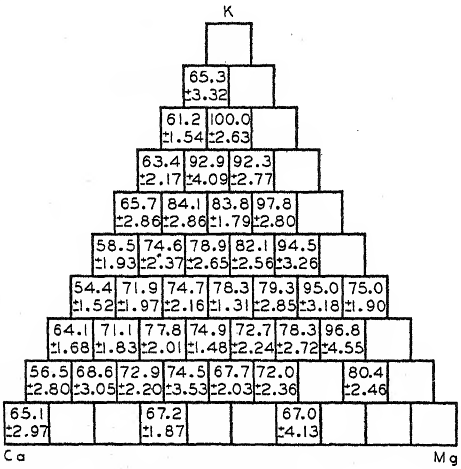

FIG. 3.—Inter-treatment comparison of stem diameters. Treatment no. 48 with stem diameter of 12.0 mm. is designated 100 and all others related to it. Treatment means, together with standard errors, given.

An inter-treatment comparison of stem diameters is given in figure 3. In general, plants from treatments supplied with small relative amounts of calcium in the nutrient medium (2.8 me./l.) had the largest stems of any in the experiment, and stem diameter was significantly less with increments of calcium supply. In the central portion of the triangle there is also a suggestion that stem diameter increases with increments of potassium supply, but no correlation with magnesium supply is evident. Stem size was significantly less than optimum in all calcium-, potassium-, and magnesium-deficient treatments. Supporting evidence for these viewpoints is furnished by a statistical analysis of the data in a manner proposed by BEESON *et al.* (1). Disregarding those treatments in which the nutrient medium was lacking in one or more ions, several significant correlations are evident between nutrient ion supply and stem diameter. If the supply of $K^+ = a$, $Ca^{++} = b$, $Mg^{++} = c$, and stem diameter $= v$; then:

$$r_{av} = +0.70^3$$
$$r_{bv} = -0.80^3$$
$$r_{cv} = +0.12.$$

In the same treatments, therefore, potassium ion supply is significantly and positively correlated with stem diameter, and calcium ion supply is significantly and negatively correlated with stem diameter. Magnesium ion supply is not correlated with stem diameter in any significant manner. Since symmetry is a condition of the experiment, the correlations are valid to illustrate trends in the data. It should be emphasized, however, that these correlations do not include data from completely deficient treatments. If such data are included, the magnitude of the correlation coefficients is slightly changed but their significance is not.

An inter-treatment comparison of the actual areas of each of the component tissue systems is given in figure 4. The data for cortex, phloem, xylem, and pith in the central portion of the triangles are very similar, showing maximum development of each tissue system in treatments supplied with small amounts of calcium (2.8 me./l.) in the nutrient medium. As greater amounts

[3] Highly significant, satisfying statistical requirements for P = 0.01.

of calcium are supplied to the plants, the area of each of the component tissue systems is decreased. Table 2 shows the correlations involving calcium ion supply. The correlation coefficients are all positive and significant. No significant relationship involving magnesium ion supply is evident. The com-

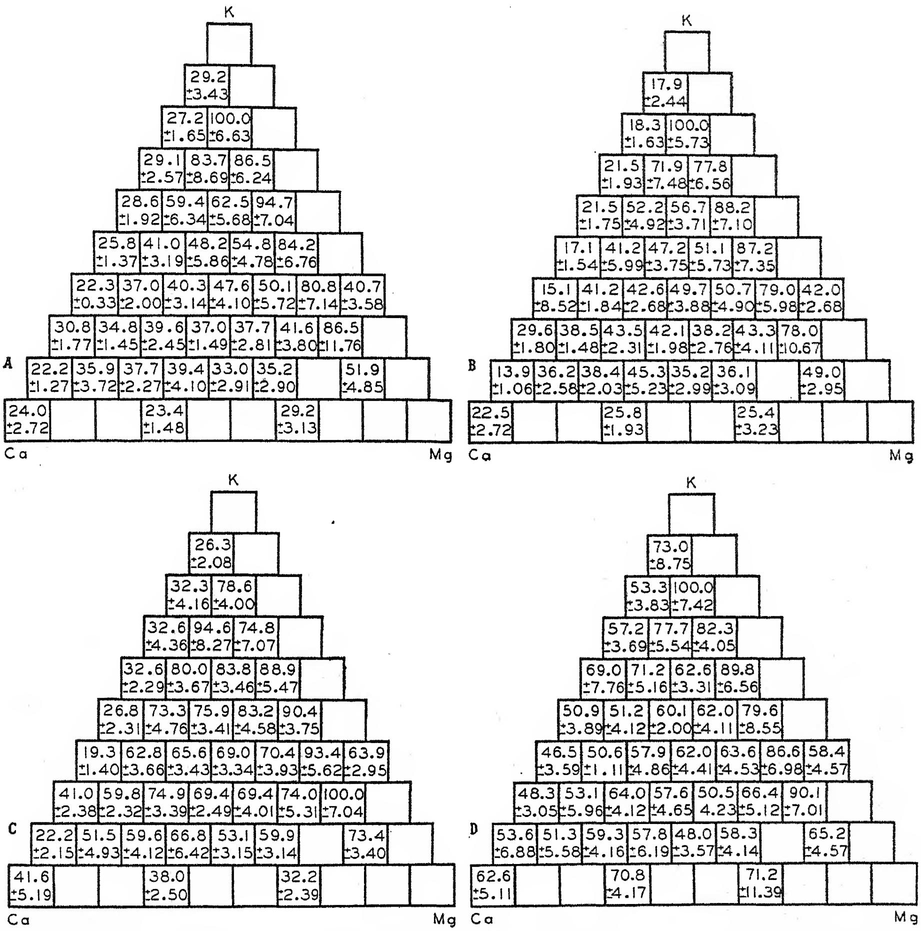

FIG. 4.—Inter-treatment comparison of actual areas of *A*, cortex; *B*, phloem; *C*, xylem; *D*, pith, in stem cross-sections. Treatment with maximum value designated 100 and all others related to it. Treatment means, together with standard errors, given.

ply and areas of each of the component tissue systems. The correlations are all negative, significant, and of the same order of magnitude. In addition, both sets of data (fig. 3; table 2) show significant increases in the area of each of the component tissue systems to be associated with increments of potassium ion supply. The correlation coefficients are all positive and significant. No significant relationship involving magnesium ion supply is evident. The complete lack of either magnesium or potassium in the nutrient medium has resulted in development of cortex, phloem, and xylem tissues which have considerably less area than the area of those in optimum treatments. The area of pith is also depressed by a lack of magnesium or potassium but to a somewhat

less extent than is the case for the other tissues. Lack of calcium supply has resulted in less pith and xylem and considerably less phloem and cortex development than was observed in optimum treatments.

TABLE 2

CORRELATION COEFFICIENTS BETWEEN CHARACTERISTICS OF STEM ANATOMY AND VARIABLES OF NUTRIENT SUPPLY

Variables of nutrient supply	Area			
	Cortex	Phloem	Xylem	Pith
K^+	+0.731*	+0.697*	+0.573*	+0.604*
Ca^{++}	−0.772*	−0.744*	−0.762*	−0.741*
Mg^{++}	+0.056	+0.062	+0.213	+0.157

* Highly significant, satisfying requirements for P = 0.01.

As would be expected, the trends with respect to stem diameters are similar to those for areas of component tissue systems. From table 3 it is evident that the areas of various tissue systems are highly significant and positively correlated among themselves and that similar correlations exist between each of the component tissue systems and stem diameter. The magnitude of the correlations is unusually high. Thus, an increase in cortical area as a result of treatment is accompanied by a corresponding increase in the area of phloem, xylem, and pith, as well as a corresponding increase in stem diameter and hence in cross-sectional area of stems. It is noticeable that the correlation coefficient in which areas of xylem and pith are involved as variables is significantly less in magnitude according to FISHER'S Z test (3) than all other coefficients.

Differences in the percentage cross-sectional area of the stem section occupied by the area of each of the constituent tissue systems are shown in figure 5. Relatively small but statistically significant differences are evident for each characteristic when treatments represented in the central portion of the triangle are compared. For instance, area of cortex varied from 17.5 to 26.9%; phloem, 12.4 to 18.0%; xylem, 22.0 to 36.5%; cortex, 29.1 to 37.3%. Since these differences are small, it is difficult to visualize trends associated with the supply of individual ions in the nutrient medium. The trends, however, can be illustrated in the data of table 4. Two contrasting situations are evident. Increases in the percentage area of either cortex or phloem are significantly and positively correlated with increments of potassium ion supply and significantly and negatively correlated with increments of calcium ion supply. No significant trends are associated with magnesium ion supply. On the other hand, increases in the percentage area of either xylem or pith are negatively and significantly correlated with increments of potassium ion supply and positively and significantly with increments of calcium ion supply. As before, no significant trends are associated with magnesium ion supply. Thus, not only stem diame-

TABLE 3

CORRELATION COEFFICIENTS BETWEEN VARIABLES IN CROSS-SECTIONS OF TOMATO STEMS

Anatomical characters	Area			Stem diameter
	Phloem	Xylem	Pith	
Area*				
Cortex	+0.974†	+0.813†	+0.820†	+0.962†
Phloem	....	+0.871†	+0.769†	+0.978†
Xylem	....	....	+0.523†	+0.915†
Pith	....	....	....	+0.800†

* Actual area of indicated tissue system in stem cross-section.

† Highly significant, satisfying requirements for P = 0.01.

ter and the actual area of each of the component tissue systems, but the relative area of each tissue system in the cross-section as well, are significantly correlated with both potassium and ment of cortex, phloem, and xylem but accentuates the relative development of pith tissue. This fact should support a negative relationship between the relative area of cortex, phloem, or xylem

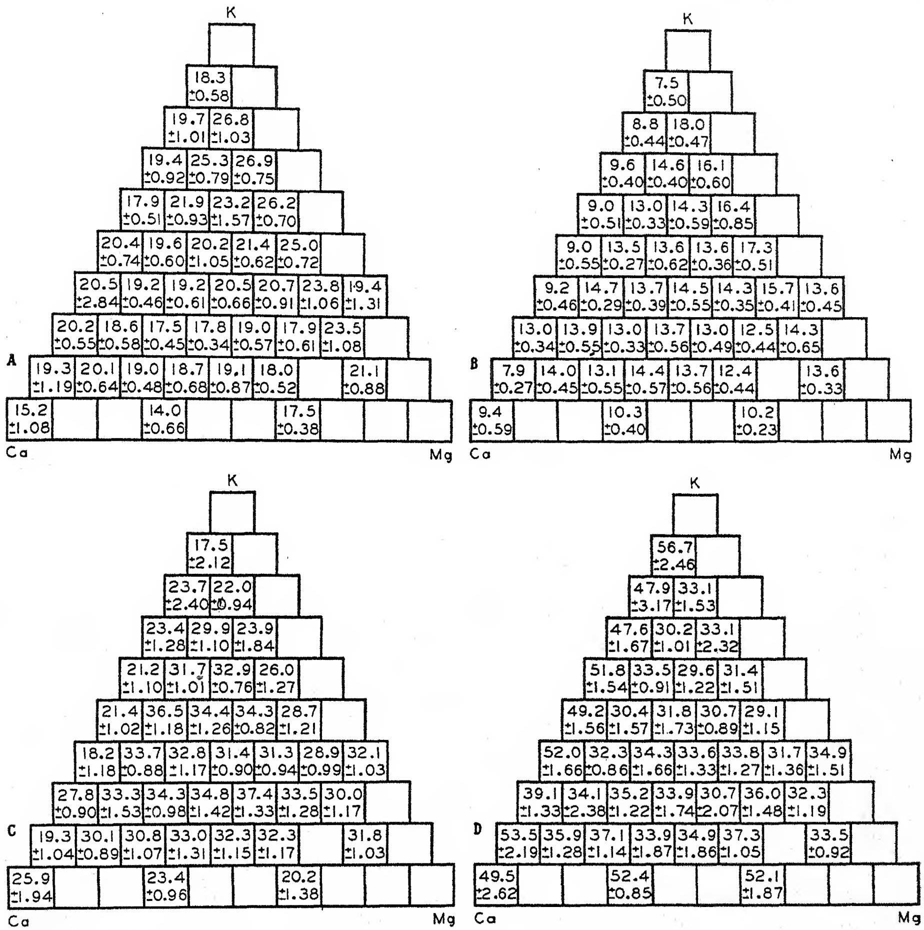

FIG. 5.—Percentage cross-sectional area of stem section occupied by area of *A*, cortex; *B*, phloem; *C*, xylem; *D*, pith. Treatment means, together with standard errors, given.

calcium ion supplies. However, the sign and magnitude of the correlation coefficients differ, depending on the characteristic under consideration.

A complete lack of potassium or magnesium ions in the nutrient medium subsequent to the seedling stage depresses to varying extents the relative development and pith. That such is actually the case is shown in table 5. Highly significant and negative correlation coefficients between percentage cortex, phloem, or xylem and percentage pith are evident. Statistically the magnitude of the correlation coefficient involving percentage cortex is significantly less than either of

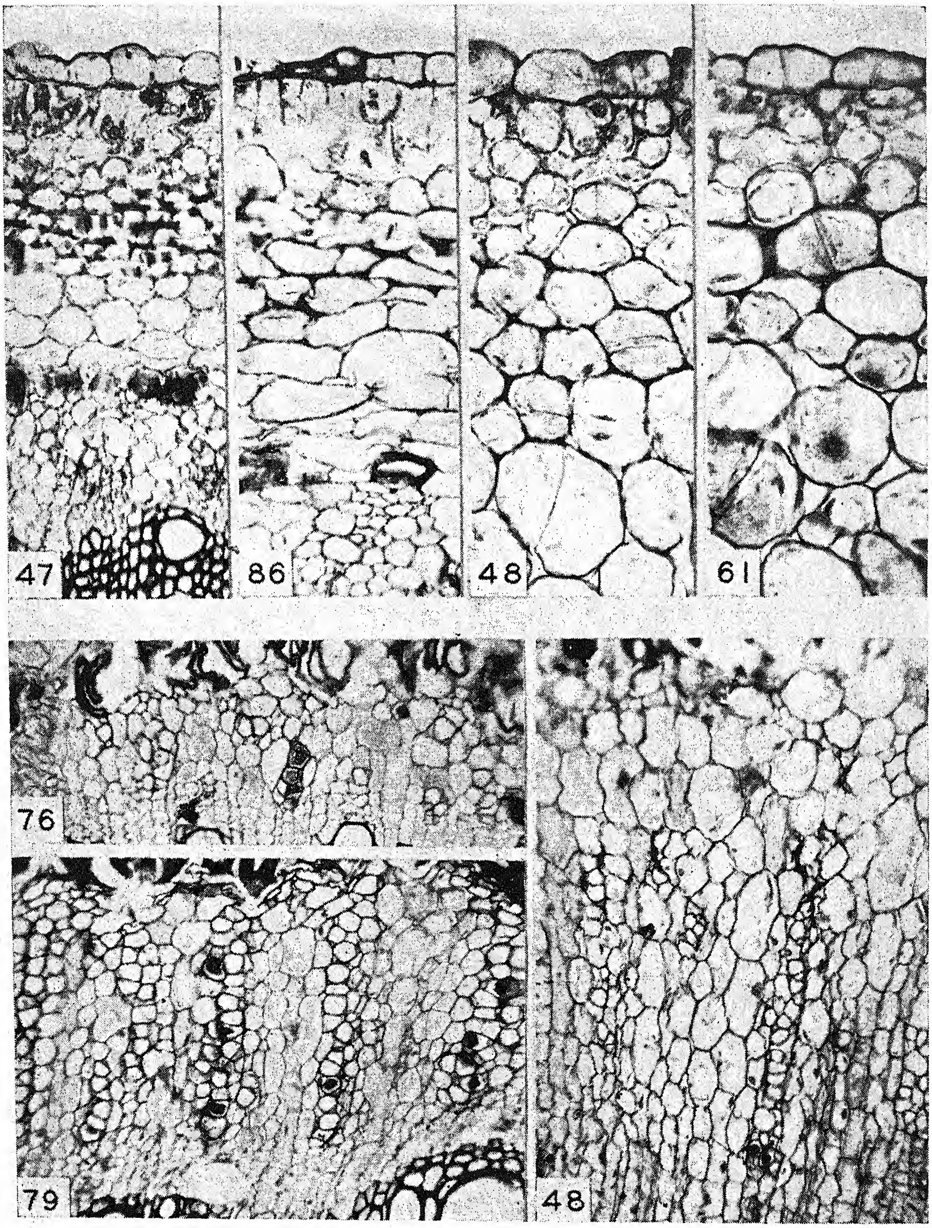

FIG. 6.—Top, series of transections of cortical sectors (external to medium-sized vascular bundles) in stems from indicated treatments; × 128. Bottom, series of transections of external phloem of comparable vascular bundles in stems from indicated treatments; × 141.

those involving percentage phloem or xylem. This may in part be due to the relatively small variation between treatments with respect to percentage cortex. Although the actual area of each of the component tissue systems increases with

TABLE 4

CORRELATION COEFFICIENTS BETWEEN CHARACTERISTICS OF STEM ANATOMY AND VARIABLES OF NUTRIENT SUPPLY

Variables of nutrient supply	Percentage area			
	Cortex	Phloem	Xylem	Pith
K^+	+0.778*	+0.607*	−0.520*	−0.602*
Ca^{++}	−0.710*	−0.479*	+0.467*	+0.506*
Mg^{++}	−0.059	−0.127	+0.049	+0.090

* Highly significant, satisfying requirements for P = 0.01.

increased stem diameter, the relatively greater development of vascular tissue (and to a less extent of cortical tissue) is made at the expense of pith. Percentage phloem is significantly and positively correlated with both percentage cortex and percentage xylem, although the magnitude of the coefficients is not large. No significant correlation between percentage cortex and percentage xylem is evident.

Extreme variation in the organization, size, and degree of differentiation of constituent cells in each tissue system was evident. Figures 6 and 7 show extremes observed in the development of cortex, external and internal phloem, and pericycle. For convenience, each photomicrograph is labeled with its treatment number, which will be used for reference in the following discussion.

The development of cortical chlorenchyma was clearly related to the supply of magnesium ion in the nutrient medium. In all magnesium-deficient treatments, two to five layers of small cells densely packed with chloroplasts were present immediately beneath the epidermis (fig. 6, no. 47). The development of chlorenchyma in magnesium-deficient treatments was not altered by variations in the supply of potassium and calcium to the plant. In treatment 48, supplied with 2.8 me./l. of magnesium, all cortical cells were larger than in magnesium-deficient treatments, but the development of chlorenchyma was relatively less extensive, in that the number of cell layers was less. In treatment 61, supplied with 14.2 me./l. of magnesium, chlorenchyma consisted of one to two layers of cells, and cortical tissue was predominantly large, succulent parenchyma. Thus, as magnesium supply to the plant was increased, the number of cell layers comprising chlorenchyma was less, and the number—and to some extent size—of parenchymatous cells adjacent to the endodermis increased. In all potassium-deficient treatments the chlorenchyma

TABLE 5

CORRELATION COEFFICIENTS BETWEEN VARIABLES IN CROSS-SECTIONS OF TOMATO STEMS

Anatomical characters	Percentage area		
	Phloem	Xylem	Pith
Percentage*			
Cortex	+0.649†	−0.050	−0.523†
Phloem	....	+0.551†	−0.904†
Xylem	....	....	−0.812†

* Percentage cross-sectional area of stem section occupied by area of indicated tissue system.

† Highly significant, satisfying requirements for P = 0.01.

was restricted to one to two cell layers, and the cells were columnar in shape, with the number of plastids minimized (no. 86).

On the other hand, collenchyma was related primarily to calcium supply. In

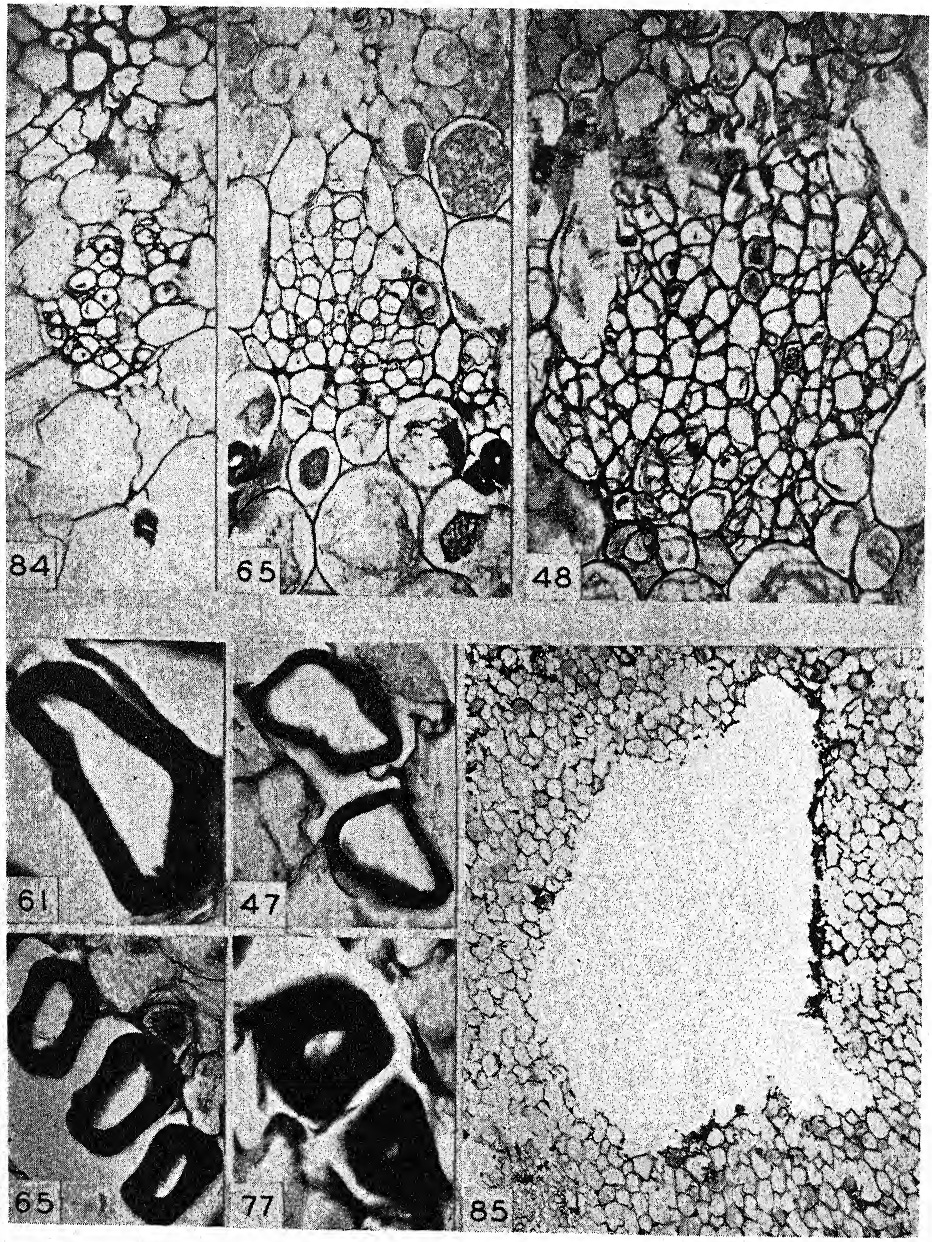

FIG. 7.—Top, series of transections showing internal phloem centrad to primary xylem points of comparable vascular bundles in stems from indicated treatments; × 192. Bottom left, representative pericyclic fibers in stem cross-sections from indicated treatments; × 432. Bottom right, transection through pith in stem from indicated treatment; × 20.

treatments supplied with 2.8 me./l. of calcium, collenchyma consisted of one to two layers of large cells with little or no thickening of the cell walls (nos. 48 and 61). As calcium supply was increased, smaller cells were evident, but the number of cell layers comprising collenchyma as well as the relative wall thickness increased. In all potassium-deficient and magnesium-deficient treatments collenchymatous cells were the smallest of any in the experiment, but cell walls were comparatively thickened (no. 47).

The characteristics of external phloem tissue appeared to be most strikingly associated with potassium supply, although some influence of calcium supply was apparent. In treatment 48 (fig. 6), which was supplied with large amounts of potassium, secondary phloem consisted primarily of large succulent parenchymatous cells with relatively infrequent sieve tubes and companion cells. As potassium supply was successively less, a greater differentiation of parenchyma into sieve tubes and companion cells was evident. The maximum relative development of sieve tubes and companion cells occurred in treatments supplied with low amounts (2.8 me./l.) of potassium (no. 79) and relatively high amounts of calcium. In other words, low potassium supply was associated with extensive differentiation of secondary phloem parenchyma into sieve tubes and companion cells, but within these treatments high calcium and low magnesium supplies were associated with maximum differentiation, and this condition was evident at each level of potassium supply. In treatments at the high-calcium apex of the triangle, it was evident that some parenchyma cells of secondary phloem in stem sections were packed with crystals—supposedly calcium oxalate (nos. 76 and 79). The number of crystal-containing cells and the amount of crystals in each cell increased with increments of calcium supply. In all magnesium-deficient treatments secondary phloem consisted primarily of parenchyma cells with isolated groups of three to five sieve tubes and companion cells. Some crushing of primary phloem was evident (no. 76). There was large variation in secondary phloem development in potassium-deficient treatments. The range was from large succulent parenchyma with small isolated groups of sieve tubes and companion cells in treatments supplied with relatively low amounts of calcium and high amounts of magnesium to highly organized sieve tubes and companion cells with sparse parenchymatous cells in treatments supplied with relatively high calcium and low magnesium.

The most extensive internal phloem development—using number of internal phloem bundles and size and number of sieve tubes and companion cells in each bundle as criteria—occurred in treatments supplied with 2.8 me./l. of magnesium (fig. 7, no. 48). Less extensive internal phloem development was associated with increased magnesium supply (no. 65). At any given level of such supply, however, the most extensive internal phloem development occurred in treatments supplied with relatively large amounts of potassium and low amounts of calcium. A complete lack of any macronutrient cation in the nutrient medium—magnesium, potassium, or calcium—resulted in minimum amounts of internal phloem (no. 84).

The largest internal and external pericyclic fibers with relatively thin walls occurred in treatments supplied with 2.8 me./l. of calcium (no. 61). As calcium supply was increased, the size

of fibers decreased and the relative wall thickness increased (no. 65). Maximum wall thickness and minimum cell size were associated with high calcium supply (no. 77). At any given level of calcium, no effect of magnesium or potassium supply was evident. Medium to large-sized fibers with minimum wall thickness were observed in treatments lacking one or more ions in the nutrient.

TABLE 6

RELATIVE COMPARISON OF EFFECTS OF THREE CONDITIONS OF NUTRIENT SUPPLY ON CELLULAR CHARACTERISTICS IN CROSS-SECTIONS OF TOMATO STEMS

Character	Treatment number		
	77 (high calcium)	48 (high potassium)	83 (high magnesium)
Cortex			
Chlorenchyma			
No. cell layers	++*	+++	+
Plastid development	++	+++	+
Collenchyma			
No. cell layers	+++	+	++
Cell size	+	++	+++
Wall thickness	+++	+	++
Cortical parenchyma			
No. cell layers	+	++	+++
Cell size	+	+++	++
Pericyclic fibers			
Cell size	+	+++	+++
Wall thickness	+++	+	+
External phloem			
Cell size	+	+++	+++
Relative amount of parenchyma	+	+++	++
Relative amount of sieve tubes and companion cells	+++	+	++
Crystal inclusions in parenchyma	+++	....	....
Internal phloem			
Cell size	++	+++	+
No. strands	++	+++	+
No. cells per strand	++	+++	+

* Development: + = minimum; ++ = intermediate; +++ = maximum.

Several distinguishing characteristics of pith cells were evident. In all potassium-deficient treatments, complete disintegration of central pith cells had occurred, while the remaining cells were small and thin-walled. The small size of pith cells in all magnesium-deficient and calcium-deficient treatments was evident, but no disintegration of central cells occurred. Some variation in size and wall thickness of pith cells in treatments in the central portion of the triangle was evident, but the differences could not be clearly correlated with variations in nutrient supply. However, some parenchymatous cells from treatments supplied with relatively large amounts of calcium were filled with granular inclusions. The occurrence of these inclusions in pith cells was closely correlated with their occurrence in phloem parenchyma.

Certain trends with respect to cellular anatomy are thus clearly correlated with nutrient supply. These trends are crudely represented in table 6. In this table three conditions of nutrient supply were arbitrarily established, and the responses associated with the complete lack of any ion in the nutrient medium were disregarded, since they would have little practical significance. It should be recognized that plants in treatment 77, denoted "high calcium," were supplied with relatively low amounts of potassium and magnesium ions in the nutrient. The responses noted are therefore associated with the relative supply of all three ions, rather than with the supply of any single ion.

In this experiment, significant and large differences occurred between treatments with respect to stem diameter and the actual area of each of the component tissue systems in the stem sections. Small but significant differences between treatments in the relative pro-

portions of the tissue systems in the stem were also evident. In a previous experiment (6), the actual area as well as the percentage area of certain tissue systems (notably vascular tissue) was correlated with the vegetative growth and fruitful conditions of the plant. In that experiment, maximum vegetative growth and maximum fruitfulness occurred in identical treatments. Since in the present experiment maximum vegetative growth occurred in treatments supplied with relatively small amounts of calcium, while maximum fruitfulness was obtained in treatments supplied with relatively large amounts of calcium (4), it is of interest to examine similar correlations. The results of the correlation analysis are given in table 7.

These data show stem diameter and the actual area of each of the component tissue systems, except pith, to be positively and significantly correlated with characteristics used as criteria of vegetative growth. This means that each unit increase in stem diameter or in the cross-sectional area of cortex, phloem, or xylem was accompanied by a unit increase in the vegetative growth of the plants. However, similar anatomical changes could not be correlated in any significant manner with fruitful conditions. The area of the pith was not significantly correlated with growth of the vine but was positively correlated with growth of the roots and negatively with fruit production. The magnitude of those correlations involving area of xylem and growth of the vines as variables is relatively high.

Changes in the relative proportions of each of the constituent tissue systems were significantly correlated with the vegetative and fruitful condition of the plant. Increased relative proportions of cortex, xylem, and phloem in stem sections were significantly correlated with

TABLE 7

CORRELATION COEFFICIENTS BETWEEN ANATOMICAL CHARACTERISTICS OF TOMATO STEMS AND CHARACTERS USED AS CRITERIA OF GROWTH AND FRUITFULNESS

Anatomical characters	Growth				Fruitfulness		
	Ht. of vine	Fresh wt. of vine	Dry wt. of vine	Dry wt. of roots	Total no. fruit/vine	Fresh wt. fruit/vine	Ave. fresh wt. mature fruit
Percentage*							
Cortex	+0.613‡	+0.566‡	+0.625‡	+0.813‡	−0.260	−0.352	−0.350
Phloem	+0.686‡	+0.824‡	+0.863‡	+0.850‡	+0.320	+0.259	+0.139
Xylem	+0.518‡	+0.564‡	+0.645‡	+0.323	+0.879‡	+0.784‡	+0.639‡
Pith	−0.771‡	−0.828‡	−0.916‡	−0.765‡	−0.584‡	−0.470‡	−0.338
Area†							
Cortex	+0.645‡	+0.683‡	+0.763‡	+0.891‡	−0.185	−0.293	−0.383
Phloem	+0.662‡	+0.761‡	+0.825‡	+0.905‡	−0.039	−0.133	−0.247
Xylem	+0.771‡	+0.810‡	+0.930‡	+0.815‡	+0.347	+0.195	+0.044
Pith	+0.259	+0.362	+0.400	+0.596‡	−0.502‡	−0.565‡	−0.674‡
Stem diameter	+0.665‡	+0.745‡	+0.833‡	+0.875‡	−0.008	−0.130	−0.274

* Percentage cross-sectional area of stem section occupied by area of indicated tissue system.
† Actual area of indicated tissue system in stem cross-section.
‡ Highly significant, satisfying requirements for P = 0.01.

increased vegetative growth. The magnitudes of all correlation coefficients involving percentage phloem as a variable are significantly higher as measured by FISHER's Z test (3) than corresponding coefficients involving percentage cortex or percentage xylem. Since the relatively greater increases in the percentage cortical and vascular tissue in the cross-sections were apparently made at the expense of pith (table 5), the correlations involving percentage pith and vegetative growth characteristics as variables are all negative with respect to sign. However, a different situation existed with respect to fruitfulness. The relative extent of cortex and phloem in the stem sections was not significantly correlated with characteristics used as criteria of fruitfulness, although highly significant and positive correlations existed when percentage xylem was considered. Thus, vegetative growth of the plants could be significantly and positively correlated with percentage phloem, and—to a less extent—with percentage xylem, while fruitfulness could not be correlated with percentage phloem but was significantly and positively correlated with percentage xylem.

Discussion

The results of two experiments will be discussed. The first involved anatomical studies of tomato stems from plants supplied with nutrient solutions varying in the relative supply of nitrate, sulphate, and phosphate (6); the present experiment involves variations in the relative supply of calcium, magnesium, and potassium. Certain general conclusions seem warranted.

Large variations in the diameter of stem sections from the middle internode of tomato plants can be associated with differences in macronutrient ion supply to the plant. Increased stem diameter can be directly and closely correlated with increased vegetative growth in all cases. Differences in stem diameter were also directly correlated with fruitfulness in the first experiment, in which differences in the fruitful condition of the plant were the result of variations in the anion supply. In that experiment (6), increased nitrate supply was associated with increased fruit production. It is probable that the direct correlation would not hold if levels of nitrate supply sufficiently high to inhibit fruiting had been used. In the second experiment, variations in stem diameter could not be associated in any significant manner with variations in fruitfulness of the plants as a result of altered calcium, magnesium, or potassium supply.

Increased stem diameter and hence cross-sectional area of stem sections was associated with increases in the area of each of the constituent tissue systems. However, variations in the percentage cross-sectional area of each constituent tissue system could also be associated with differences in nutrient composition. Percentage cortex was relatively constant, although small but significant variations occurred in the cation triangle. Differences in the relative area of the vascular tissues could be correlated primarily with the level of potassium, calcium, or nitrate supply. Large differences in the percentage pith occurred between treatments, and percentage pith was inversely correlated with the relative amount of vascular tissue in all cases. Both percentage phloem and percentage xylem were positively and significantly correlated with vegetative growth in both triangles, but correlations involving percentage phloem were invariably significantly higher than those

involving percentage xylem. On the other hand, percentage phloem is not necessarily correlated with fruit production, although significant correlations may be obtained if variations in fruitfulness are the result of differences in nitrate supply. Percentage xylem has invariably been positively and significantly correlated with fruitfulness.

Large differences in cellular characteristics have been correlated with nutrient composition, although more experimentation seems desirable to associate the differences more closely with individual ions. These observations, however, can serve as a basis for future experimentation. For instance, such correlations as calcium ion supply with the development and cellular characteristics of collenchyma; magnesium with chlorenchyma; calcium and nitrate with pericyclic fibers; potassium, calcium, or nitrate with external phloem; nitrate with xylem; and magnesium, potassium, or nitrate supply as they can be correlated with size and structure of internal phloem strands might be useful in evaluating the nutritional status of a tomato plant.

Several anatomical considerations may be of interest. It is evident that both cell size and thickness of cell walls can be related to nutrient composition in so far as the supply of macronutrient ions is varied. With respect to such correlations involving nitrogen, the carbohydrate metabolism of the plant would seem to offer the most logical postulate if a cause-and-effect relationship exists. However, the supply of calcium ion is clearly correlated with cell size and the thickening of cell walls in collenchyma and pericycle. All treatments in the cation triangle were supplied with equal amounts of nitrate in the nutrient medium and contained equal amounts of nitrogen per unit dry weight of leaf at maturity (5). Although plants supplied with relatively large amounts of calcium made less vegetative growth, fruitfulness was materially greater than in plants supplied with relatively low amounts of calcium (4). By computation, it is evident that low-calcium plants produced less total dry weight (roots+vines+fruit) than did high-calcium plants. It seems possible that if carbohydrate utilization is a factor in the anion triangle which can be correlated with cell size and thickness of cell walls, it can also be a factor in the anion triangle. In the anion triangle, different carbohydrate-nitrogen balances may have been attained by a differential in the assimilation of nitrogen. In the cation triangle, a similar difference in the balance may have been attained by a differential in the assimilation of carbohydrate associated with altered relative cation supply.

It seems possible that the results presented here may serve as a guide for future work designed to study the influence of macronutrient elements on cellular physiology. The fact that the supply of a given element may influence the type and amount of differentiation in cell growth may indicate a clue as to the utilization of this element in the cellular metabolism. It seems possible that the plants which received a high calcium supply were more fruitful than those which received a low calcium supply because these plants developed greater amounts of phloem. On the other hand, it is just as possible that the influence of calcium is an indirect one on the utilization of carbohydrate and nitrogenous food material and that the tissue developments correlated with calcium are secondary and the result of the influence of the greater fruitfulness on these tis-

sues. It is possible that results of similar experiments may aid in a clearer understanding of the role of some of the macronutrients in cellular metabolism and differentiation.

Summary

1. An inbred strain of Bonny Best tomatoes was grown in sand culture. The effects of forty-three nutrient solutions varying in the relative proportions of macronutrient cations (calcium, potassium, and magnesium) were studied in relation to the anatomy of plant stems. Measurements of stem diameter and the actual area of each of the component tissue systems were recorded. The data were reduced and analyzed by statistical methods.

2. Great differences in stem diameter and the actual area of each of the component tissue systems were positively correlated with potassium and negatively correlated with calcium supply to the plant.

3. Significant differences between treatments occurred with respect to the relative areas of the constituent tissue systems in the stem sections. These differences could also be correlated with nutrient composition.

4. Cell size and relative thickness of cell walls in pericyclic fibers could be associated with calcium ion supply. Cellular differences were also observed in pith parenchyma, internal and external pericyclic fibers, internal and external phloem, and in cortical chlorenchyma and collenchyma. They are described and correlated with the supply of one or more ions in the nutrient medium.

5. Differences in the anatomy of the tomato stems are significantly correlated with characteristics used as criteria of vegetative growth and fruitfulness. For instance, the relative amount of phloem in stem sections is positively correlated with vegetative growth, while the relative amount of xylem is positively correlated with fruitfulness.

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LITERATURE CITED

1. Beeson, K. C., Lyon, C. B., and Barrentine, M. W., Ionic absorption by tomato plants as correlated with variations in the composition of the nutrient medium. In press.
2. Fisher, R. A., The design of experiments. Oliver & Boyd, London. 1937.
3. ———, Statistical methods for research workers. Oliver & Boyd, London. 1936.
4. Hamner, K. C., Lyon, C. B., and Hamner, C. L., Effect of mineral nutrition on the ascorbic-acid content of the tomato. Bot. Gaz. 103:586–616. 1942.
5. Lyon, C. B., Beeson, K. C., and Barrentine, M., Macro-element nutrition of the tomato plant as correlated with fruitfulness and occurrence of blossom-end rot. Bot. Gaz. 103:651–667. 1942.
6. Lyon, C. B., and Garcia, C. R., Anatomical responses of tomato stems to variations in the macronutrient anion supply. Bot. Gaz. 105:394–405. 1944.
7. Snedecor, G. W., Statistical methods. Collegiate Press, Ames, Iowa. 1938.
8. Tippett, L. H. C., Tracts for computers. Random sampling numbers. Cambridge Univ. Press, London. 1927.
9. Yates, F., The analysis of replicated experiments when the field results are incomplete. Empire Jour. Exp. Agr. 1:129. 1933.

MORPHOLOGICAL IDENTITY OF THE VELAMEN AND EXODERMIS IN ORCHIDS

CHARLES J. ENGARD

Literature review

The velamen, a specialized tissue of from one to many layers of cells covering the aerial and terrestrial roots of orchids, is separated from the cortical parenchyma by a uniseriate layer of cells which is neither the velamen nor the cortex in structure. This layer, termed endodermis, exodermis, or hypodermis by previous investigators, is composed of vertically alternately long and short cells which may be variously modified structurally and functionally.

SCHLEIDEN (11), who in 1849 named the ensheathing tissue *velamen radicum*, interpreted it as a many-layered root envelope overlying the epidermis. CHATIN (1) also held this opinion. SCHACHT (10) and OUDEMANS (9) thought that the outermost layer of cells was epidermis and that immediately below this was one component of cortex separated from the inner component by a specialized layer, the endodermis (now exodermis): LEITGEB (7) held that the velamen is developed "subsequently and directly from the epidermis by division of its cells." TREUB (13), MEINECKE (8), DE BARY (2), and HABERLANDT (4) adopted LEITGEB's concept and terminology. More recently, JEFFREY (6) and SOLEREDER and MEYER (12) referred to the velamen as a many-layered epidermis.

TREUB and DE BARY seem to be the only ones to have investigated the histogenetic origin of the velamen. The former noted in *Vanilla* and *Stanhopea* that the dermatogen which gave rise to the velamen was developed at the apex from a common initial group for the root cap and the body of the root. DE BARY found a similar situation in *Vanda furva*, but in *Oncidium* he found that "the dermatogen passes over the growing-point as a distinct layer between periblem and calyptrogen."

The textbook of anatomy currently used (3) mentions the velamen of orchids but makes no reference to its morphological identity.

The inner limiting layer of the velamen, termed endodermis by the early investigators, had a special significance to DE BARY, who referred to it as a special case of endodermis. (The latter term he applied to the tissue, adjacent to pericycle, which previously had been called "protective sheath.") HABERLANDT later referred to this endodermis as exodermis.

HAYWARD (5) records for *Linum* a periblem consisting of two layers, the outer one of which divides anticlinally to form the outermost layer of cortex or hypodermis; the inner one divides in all planes to form the remainder of the cortex. Hypodermis is thus made synonymous with exodermis.

It is apparent that several aspects of the problem of velamen ontogeny need investigation. These are (*a*) the histogenetic constitution of the root apex; (*b*) the origin of the velamen—its morphological identity; (*c*) the origin of the exodermis—its morphological identity; (*d*) the relationship between exodermis and hypodermis.

Methods

Aerial and subterranean roots were killed, fixed, sectioned in paraffin, and stained with safranin or with Delafield's haematoxylin.[1] No counterstains were used. The roots of twenty species and hybrids, representing thirteen genera of epiphytic and terrestrial orchids, were

[1] Thanks are due MAMORU ISHII for preparing more than half the slides.

investigated. These, with the number of rows of cells in the velamen, are as follows:

Aerides lawrenceae Rchb. f.*	4 rows
Arundina graminifolia Scltr. (*A. bambusifolia* Lindl.) [terrestrial]†	2
Brassavola cucullata R. Br.†	5–6
Brassavola nodosa Lindl.†	5–7
Brassia brachiata Lindl.†	10–12
Cattleya manoa†	5
Cattleya percivalliana Rchb. f.× *C. santa monica*†	4–5
Cattleya trianae Rchb. f.†	8–10
Dendrobium sp.	3–4
Epidendrum catillus Rchb. f. et Warse†	6
Epidendrum cinnabarinum Salzm. [terrestrial]‡	6
Epidendrum radicans Pav.†	6
Oncidium sphacelatum Lindl.†	8–10
Phalaenopsis schilleriana Rchb. f.†	3
Phalaenopsis sumabilis (*P. sumatrana* Korth. ×*P. amabilis* Bl.)†	2
Spathoglottis×*parsonii*×*S. plicata* Bl. [terrestrial]†	2
Sobralia macrantha Lindl. [terrestrial]	3–4
Vanda luzonica Loher†	6
Vanda parishii Rchb. f. (*Vandopsis parishii* Schltr.)†	7–8
Vanilla planifolia Andr.*	1

* Obtained from the Foster Gardens through the courtesy of COLIN POTTER.

† Obtained from plants in the Frank Atherton collection through the courtesy of O. M. KIRSCH.

‡ Obtained through the courtesy of Dr. C. P. SIDERIS of the Pineapple Research Institute. All of Honolulu.

Investigation

HISTOGENETIC CONSTITUTION OF APEX

Certain of the histogen types established by HANSTEIN and developed by JANCZEWSKI and HABERLANDT, although no longer applicable to the stem apex, will be utilized in this study. The root tips of all but six of the twenty species investigated belong to type I, in which four distinct histogens give rise to all tissues of the root. The remaining six—*Aerides lawrenceae*, *Phalaenopsis sumabilis*, *P. schilleriana*, *Vanda luzonica*, *V. parishii*, and *Vanilla planifolia*—belong to HABERLANDT's type VI, in which the tissues are derived from a common mass of cells at the root apex but in which the protoderm takes no part in root-cap formation as in type IV.

Figure 1, a portion of the root apex of *Sobralia macrantha*, is representative of the others of this histogen category. The cells of the histogens, the protoderm and its derivatives, the exodermis, and the peripheral cells of the procambium have been stippled for emphasis. The emphasis is not unduly exaggerated, for these tissues show up clearly with the single-stain procedure (fig. 3). Figure 2, *Phalaenopsis sumabilis*, shows the cells of the protoderm and its derivatives, the exodermis. There is not one distinct histogen in this root; the fundamental tissues arise from a large mass of meristematic cells. In other roots of this type there is developed a distinct calyptrogen below the embryonic mass and just outside the protoderm. The latter tissue does not contribute to the calyptra or calyptrogen.

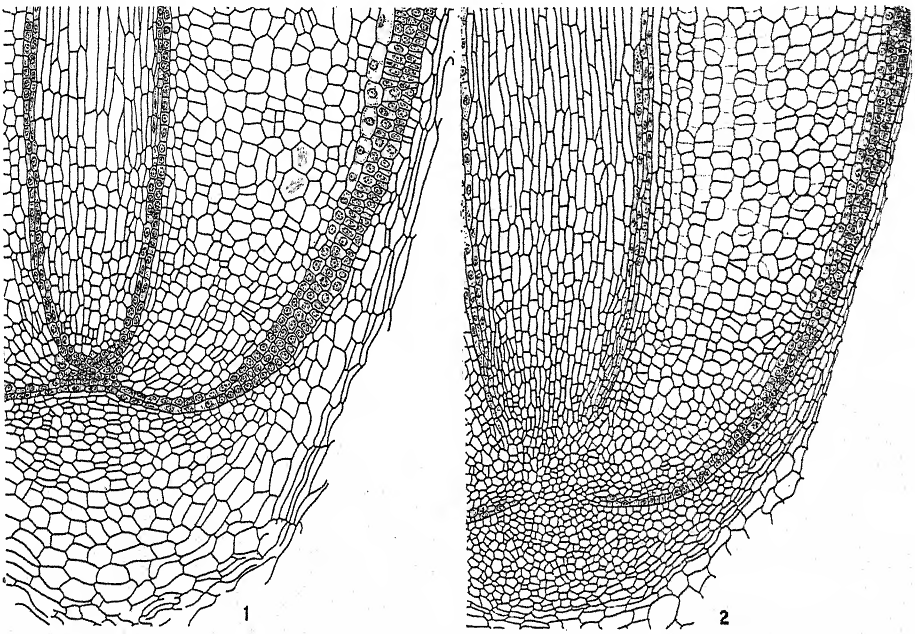
These two types of histogenetic constitution were observed by TREUB in *Vanilla* and *Stanhopea* (type VI), and by DE BARY in *Vanda furva* (type VI) and in *Oncidium* (type I). On the basis of results of the present study, apparently roots with a distinct dermatogen are the more common in orchids.

ONTOGENY OF VELAMEN

The velamen is derived from the protoderm by periclinal divisions (figs. 1, 2, 4, 5). The cells of the latter, which are alternately long and short (fig. 5), become vacuolated early, and they thereby clearly mark the internal boundary of the velamen. In *Vanilla* the velamen is only one cell in width; in *Oncidium* it is

8–10 cells in width. Root hairs are frequently developed (fig. 7).

Although there appears in the literature no general agreement of definition of epidermis, the term multiple or many-layered epidermis has often been used. Some investigators seem to prefer to limit the concept of epidermis to the outermost layer of tissue. Since in the ontogeny of the many-layered dermal system the cells are derivatives of the protoderm, they are therefore closely related histologically. The velamen is here considered *in toto* to be multiple epidermis.



FIGS. 1, 2.—Fig. 1, camera lucida diagram of portion of root tip of *Sobralia macrantha* showing type I histogens and origin of velamen and exodermis. Fig. 2, same of *Phalaenopsis sumabilis* showing type VI histogens and origin of velamen and exodermis.

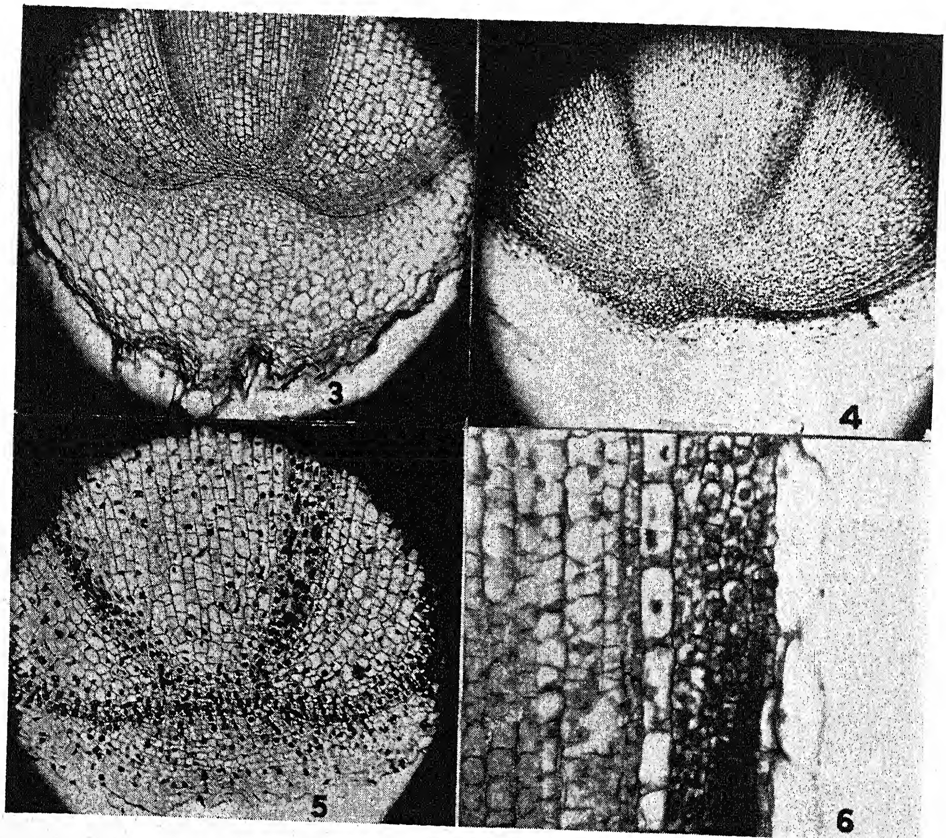
ORIGIN OF EXODERMIS

It was noted previously that the layer of cells forming the inner limit of the velamen has been considered to be (*a*) the endodermis proper, and (*b*) a special kind of endodermis called exodermis. In all roots investigated, this limiting tissue is derived from the periblem and is therefore to be considered morphologically as specialized cortical tissue (figs. 1, 2).

Owing to the lack of discrete histogens in the type VI root tips, it is impossible to establish a histogenetic origin for the exodermis in those roots. The exodermis is clearly discernible as a distinct uniseriate row of cells all the way down to the meristematic group. It is in all respects similar in its ontogeny to its counterpart in type I roots, and it should be considered at maturity as the specialized outermost layer of the cortex. The term exodermis is retained in this paper as proper terminology to apply to this tissue, since this tissue is similar to the endodermis in its ontogeny and in its

outer limiting position in the cortical tissue.

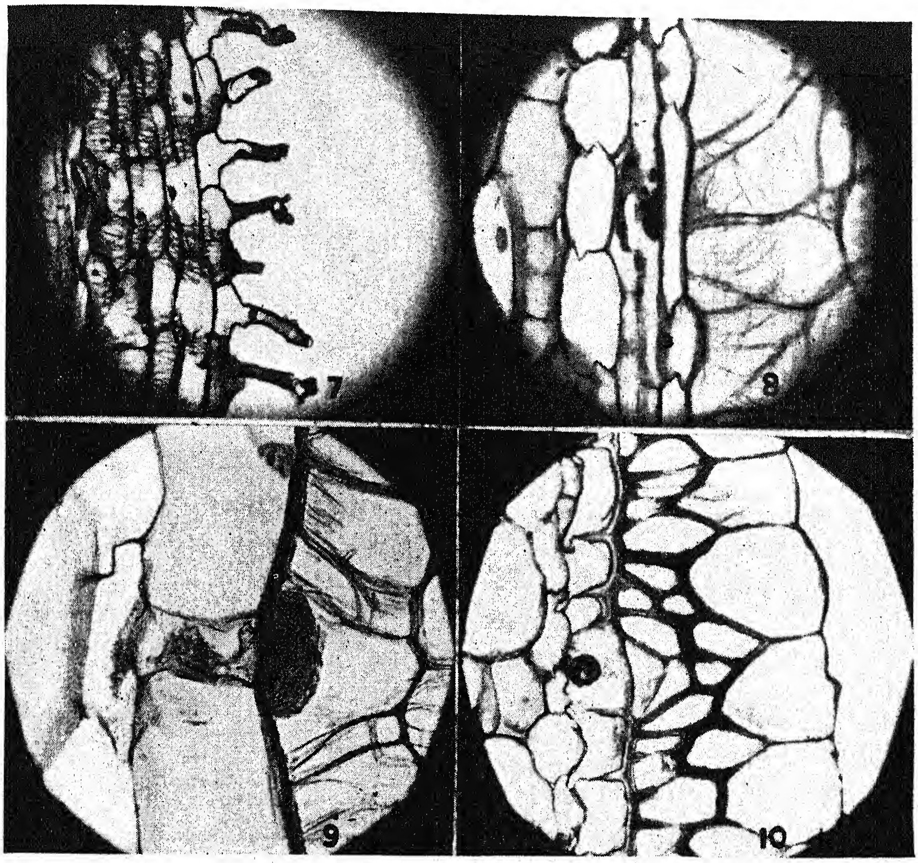
The structure of the mature exodermis is much more specialized than that of the endodermis. Early in the ontogeny of the exodermis, vertical rows of cells are alternately elongate and short (fig. 6). In the early investigations these were called long endodermis cells (*lange Endodermiszellen*) and short endodermis cells (*kleine Endodermiszellen*); later the short cells were termed passage cells, similar to their counterparts in the endodermis of certain roots. The long cells develop thick secondary walls on the radial, and especially on the outer tangential, walls. The passage cells remain thin-walled. The protoplasm of the latter cells is dense, and the nucleus is very large and prominent; but in the long cells the protoplasm is sparse and the nuclei less prominent.



FIGS. 3–6.—Fig. 3, root tip of *Sobralia macrantha*. Fig. 4, root tip of *Vanda luzonica*. Fig. 5, root tip of *Epidendrum cinnabarinum*. Fig. 6, longitudinal view of developing velamen (at right) and prominent exodermis showing long and short cells.

The cells of the velamen are variously thickened, but those adjacent to the passage cells fall, according to the species, into one of several types. In the simplest kind the velamen cells next to the passage cells are similar to the other cells of the velamen, as in *Brassia brachiata* (fig. 8); there is no specialization. In

species of *Cattleya*, the cells adjacent to the passage cells, as in *Brassia*, are not thickened, or are only slightly thickened, as in *Cattleya trianae* (not here illustrated). The most highly developed vela-



FIGS. 7–10.—Fig. 7, portion of root-hair group developed from outermost velamen cells in *Vanda luzonica*. Fig. 8, portion of exodermis showing thin-walled passage cells and velamen of *Brassia brachiata*. Fig. 9, passage cell and fibrous body of *Sobralia macrantha*. Fig. 10, thin-walled passage cells of *Dendrobium* sp. showing cover cells in velamen.

men cells adjacent to passage cells are found in *Sobralia macrantha* (fig. 9). These have been depicted diagrammatically by MEINECKE (8) and by HABERLANDT (4). A large hemispherical pad of fibrous material develops on the inner tangential walls of the velamen cell (or cells) adjacent to the passage cell. This was termed by MEINECKE *Stabkörper*, or fibrous body, and that name is still used. The body is composed of very minute fibrous ridges and rods which become enmeshed. In certain orchids, especially those with a velamen comprised of only a few layers of cells, the thin-walled passage cell is covered by a group of wedge-shaped cells, the walls of which are much thinner than adjacent velamen cells (fig. 10, *Dendrobium*). HABERLANDT discusses the structure of certain aerial roots, among them orchids. In *Taeniophyllum zollingeri* he has described a structure comprised of three

distinct parts: a mass of velamen cells attached to a single or to two or three thin-walled, air-containing exodermal cells which in their turn abut one or more rows of special rounded parenchymatous elements, the latter of cortex. This apparatus he refers to as a pneumathode. None were encountered in the roots investigated in the present study.

Relationship between exodermis and hypodermis

It was noted earlier in this paper that the term exodermis replaced endodermis, and that the latter term was then applied to the inner limiting layer of cortex. Hayward referred to the outer limiting layer (exodermis) as hypodermis. De Bary, and later Eames and MacDaniels, described the hypodermis as essentially sheets or groups of tissue, often sclerenchyma, which form a supporting or protective layer adjacent to the epidermis. Hypodermis thus defined refers to spacial relationship with the epidermis and not to ontogenetic relationship with the cortex, and could more appropriately be referred to as cortical sclerenchyma, etc. The exodermis should not be considered in the same category as hypodermis.

Summary

1. Historical concepts of the velamen and exodermis are reviewed.

2. Twenty species and hybrids, representing thirteen genera of orchids, were investigated to determine the origins of the velamen and exodermis.

3. Fourteen orchid root tips belong to the category of histogens known as type I; the other six belong to Haberlandt's type VI.

4. The velamen is derived from the protoderm. It is a multiple epidermis.

5. The exodermis is the specialized outermost layer of the cortex. It corresponds, in its limiting position and similar ontogeny, to the endodermis.

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Literature cited

1. Chatin, A., Anatomie des plantes aériennes de l'ordre des Orchidées. I. Mémoire: Anatomie des racines. Mém. Soc. Sci. Nat. Cherbourg 4:5–18. 1856.
2. De Bary, A., Comparative anatomy of the vegetative organs of the phanerogams and ferns. Engl. transl. by F. O. Bower and D. H. Scott. Oxford. 1884.
3. Eames, A. J., and MacDaniels, L. H., An introduction to plant anatomy. New York. 1925.
4. Haberlandt, G., Physiological plant anatomy. Engl. transl. by Montagu Drummond. London. 1914.
5. Hayward, H. E., The structure of economic plants. New York. 1938.
6. Jeffrey, E. C., The anatomy of woody plants. Chicago. 1917.
7. Leitgeb, H., Ueber die Luftwurzeln der Orchideen. Ann. Mag. Nat. Hist. 14:159–160. 1864. (Also Ber. Akad. wiss. Wien, Anzeiger 1:87–88. 1864.)
8. Meinecke, E. P., Beiträge zur Anatomie der Luftwurzeln der Orchideen. Inaugural-Dissertation, Univ. Heidelberg. Munich. 1894.
9. Oudemans, C. A., Ueber den Sitz der Oberhaut in den Luftwurzeln der Orchideen. Verhand. Kon. Akad. Wetensch. Amsterdam 9:4–32. 1861.
10. Schacht, H., Beiträge zur Anatomie und Physiologie der Gewachse. I: 285, 1856; II: 168, 1859.
11. Schleiden, M. J., Grundzüge der wissenschaftlichen Botanik. 4:195. 1861.
12. Solereder, H., and Meyer, F. J., Systematische Anatomie der Monokotyledonen. VI. Scitamineae-Microspermae. Berlin. 1930.
13. Treub, M., Le Meristeme primitif de le racine dans les Monocotylédones. Leiden. 1876.

NATURE AND RATE OF DEVELOPMENT OF ROOT SYSTEM OF APOCYNUM CANNABINUM[1]

JOHN C. FRAZIER

Introduction

This is the fourth of a series of reports on growth habits of noxious perennial weeds of central United States being conducted by the Kansas Agricultural Experiment Station. A study (2) of the nature and rate of development of field bindweed established the scope and methods of procedure. The second investigation (3) dealt with hoary cress, and the third (4) with Russian knapweed. The present study is concerned with dogbane, *Apocynum cannabinum* L., a noxious weed of potential significance rivaling that of field bindweed, hoary cress, and Russian knapweed, particularly when its widespread distribution and its abundance are considered. Its noxiousness is not usually appreciated. GATES (5) states that it is one of the weeds of statewide distribution which are increasing markedly in abundance in Kansas.

Environmental conditions and methods

SOIL DATA.—All plants[2] considered in this paper were taken from soil, the profile for the upper 60 inches of which was described by FRAZIER (3). The lower 24 inches of this 7-foot profile, which was mellow throughout, had the same structure as the 22–60-inch interval. The entire profile description was supplied by Dr. J. C. HIDE of the Kansas Agricultural Experiment Station.

METEOROLOGICAL DATA.—The monthly and annual temperature and precipitation data for the first 10 months of 1943 compared with the long-time average of these factors are given in table 1. The summer of 1943 had temperatures of 100° F. or higher 9 days as compared with 38 such days in 1937, when the comparable study was made on field bindweed; 15 in 1941, when the comparable study was made on hoary cress; and 5 in 1942, when the comparable study was made on Russian knapweed. The average annual number of days having temperatures of 100° F. or higher during the 50 years, 1889–1938, is 15. The average monthly precipitation for the first 10 months of 1943 was 3.18 inches, as compared with the monthly average of 2.86 for these months during the 83-year period, 1858–1940. Some of the precipitation for the first 10 months of 1943 fell as heavy rains. There were three 24-hour periods in June with 2.93,

TABLE 1

MONTHLY AND ANNUAL TEMPERATURES (IN ° F.) AND PRECIPITATION (IN INCHES) AT MANHATTAN, KANSAS, 1943

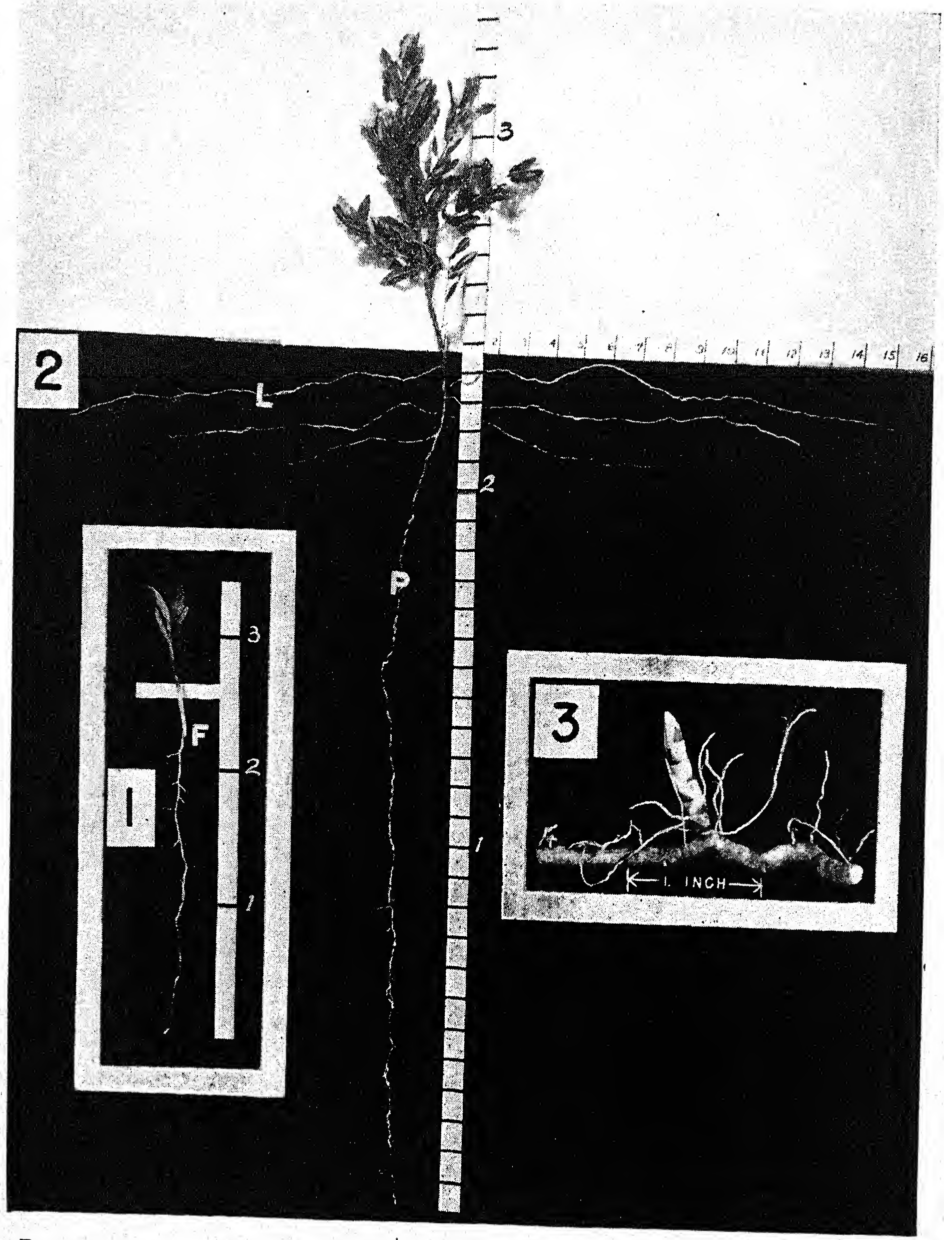
MONTH	TEMPERATURE		PRECIPITATION	
	Mean*	85-year average (1856–1940)†	Total*	83-year average (1858–1940)†
Jan.	27.8	27.0	0.22	0.75
Feb.	39.6	31.5	1.15	1.14
Mar.	40.0	42.5	0.98	1.48
Apr.	58.6	54.5	1.15	2.67
May	62.0	64.6	3.14	4.31
June	75.7	74.5	12.28	4.50
July	79.5	79.5	4.68	4.25
Aug.	83.4	77.5	1.71	3.89
Sept.	67.6	69.3	4.23	3.44
Oct.	56.6	56.5	2.29	2.16
Annual mean or total	55.2	54.2	34.13	30.97

* Meteorological data obtained from U.S. Weather Bureau, Kansas section.

† Computed by Dr. A. B. CARDWELL of Kansas State College.

[1] Contribution no. 455, Department of Botany, Kansas Agricultural Experiment Station.

[2] The term plant as used in this paper refers to all the growth from a single seed. The term shoot is applied to the individual leafy stems commonly called plants in control studies.



FIGS. 1–3.—Stages in development of plants of dogbane: Fig. 1, seedling 2 weeks after emergence showing first pair of true leaves above the two cotyledons; *F*, root-stem transition zone. Scale in inches. Fig. 2, young plant 10 weeks after seedling emergence showing relation of primary vertical root (*P*) to permanent lateral roots (*L*) of first order (growing tips not obtained). Scale in feet and inches. Fig. 3, piece of permanent lateral root bearing short rhizome; latter had grown approximately one-third of distance to soil surface. Note scale leaves on rhizome.

2.65, and 1.82 inches, respectively, one in July with 1.48 inches, and one in September with 1.52 inches.

METHODS.—The plants were grown on a small plot of land free of other noxious weeds and of sodium chlorate. The soil is a fairly typical Geary silt loam representative of the wind-deposited soil of the upland of this locality. The plot had been cultivated a decade earlier but had not been disturbed recently.

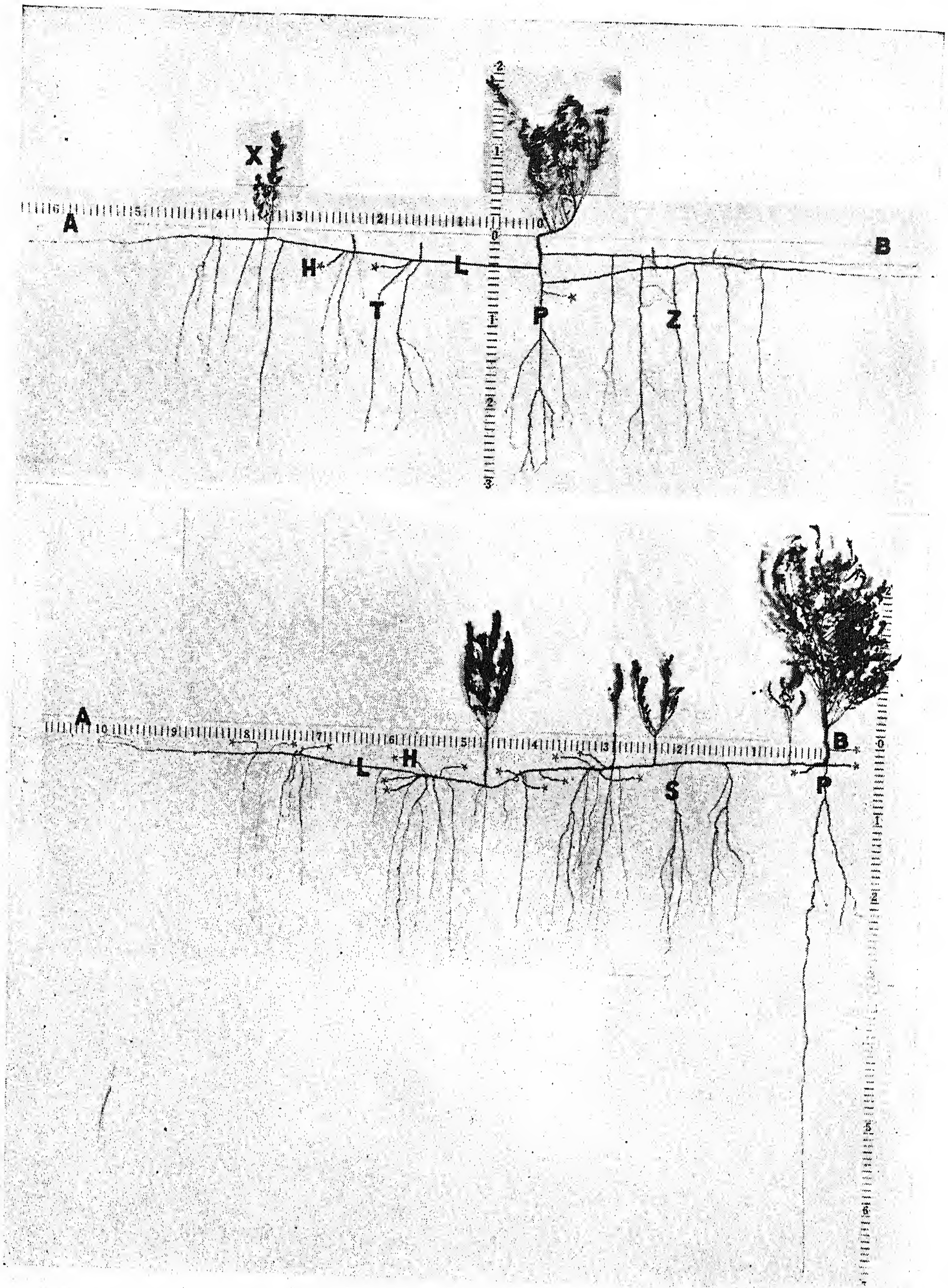
The first planting was made on April 2, 1943. Three seedlings emerged from this planting, but only the one which emerged on April 15 lived. It was excavated after 7 months of growth and was designated plant no. 8. A second planting was made on May 3 and emergence began May 14. On May 17 seven seedlings emerged. The plant developing from one was excavated after 5 months of growth. It was designated plant no. 7. A third planting was made on May 15. Emergence began May 26, and on May 27, eleven seedlings emerged at as many points. Plants developing from six of these were used for plants 1–6, inclusive. In each planting, twenty seeds, which had been kept moist at 34° F. for at least 14 days, were placed $\frac{1}{4}$ inch below the surface at each of eighteen points which were so spaced that eight of the plantings were 15 feet distant from any other and the remainder were separated by 9 feet. All the nineteen seedlings emerging on the three dates (April 15, May 17, and May 27) were retained, but all others, including those emerging after May 27, were removed. The area was kept free of all other vegetation, so that the only competition for water and plant nutrients, if any existed, was among the plants of dogbane.

The root systems were excavated by modification of the trenching method developed by WEAVER (10) as described by FRAZIER (2). Efforts were made to keep the root system as one organic entity; if a root broke, the two ends were immediately tied together. Measurements of the plant parts were made and recorded, so that as illustrated they occupied the same relationship, one part to another, as they did in the soil, except that in figures 1, 2, 4, and 5 all lateral roots are arranged in one plane. While as many as possible of the finer roots were obtained, it is not contended that a large portion of them was secured.

Observations

The rate of plant development under known soil and climatic conditions was observed by means of eight plants excavated 1, 2, 4, 5, 8, 10, 20, and 28 weeks after seedling emergence. The dates of excavation were June 3, June 10, June 24, July 1, July 22, August 5, in early October, and in early November, 1943. The plants are designated 1–8, inclusive. Figures 1, 2, 4, and 5 show the vertical penetration and the radial spread of roots of representative plants. Data of the eight excavations are given in table 2.

By 10 weeks after emergence the general plan of the root system was becoming established (fig. 2). Plant no. 7, taken the twentieth week after emergence (fig. 4), showed particularly well the gross morphological nature of the root system. A taproot rapidly penetrated directly downward from the germinating seed. It was a primary root in order of development and vertical in position; hence, it is designated the primary vertical root (fig. 4*P*). Many branch roots arose throughout the length of this taproot, most of them small feeding roots. A few, probably those more favorably situated in relation to soil moisture and plant nutrients, grew extensively and became per-



FIGS. 4, 5.—Plant parts arranged to show essentially same relationship as in the soil. *A-B*, ground line. Scales in feet and inches. Fig. 4 (above), complete plant 20 weeks after seedling emergence: *P*, primary vertical root; *L*, permanent lateral root of first order (star at right of center indicates where permanent lateral root of first order was removed for clarity); *H*, permanent lateral root of second order (two stars at left indicate permanent laterals of second order removed); *Z*, secondary vertical root which arose on lower side of permanent lateral root and grew downward; and *T*, secondary vertical root which arose on side of permanent lateral root and grew horizontally approximately 7 inches before turning downward. Note large root system produced by the two shoots, smaller of which emerged only 5 weeks earlier. Fig. 5 (below), portion of plant 28 weeks after seedling emergence. Primary vertical root (*P*) subtending extensively developed original shoot; stubs of three permanent lateral roots of first order indicated by stars immediately to right and left of primary vertical root at ground line; and approximately 70% of root and all of shoot development of longest, typical, permanent lateral root of first order (*L*) showing the four rhizomes, each bearing a shoot, the twenty-nine secondary vertical roots (*S*), and stubs of thirteen permanent lateral roots of second order (*H*), each designated by star indicating a root removed.

manent laterals (fig. 4*L*). They are designated permanent lateral roots of the first order. These roots tended to grow as radii about the primary vertical root in a plane parallel to the soil surface in the upper foot, more commonly in the upper 7 inches of the soil. They gave rise to branch roots over any part of their growth. Most of these were small feeding roots. However, some arose which appeared similar to these feeding roots. They developed into branch lateral roots, which are designated permanent lateral roots of the second order (figs. 4*H*, 5*H*). They grew horizontally at right angles to the root on which they arose for a distance of approximately 2–8 inches, then the majority grew horizontally away from the primary vertical root. In a similar manner permanent lateral roots of the third order arose on those of the second, and those of the fourth order on those of the third, etc. This branching tended to develop in the area between the permanent laterals of the first order. The result was that the plant occupied a much higher proportion of the ever-increasing area, somewhat circular in shape, than it would have done otherwise. The permanent laterals of the first order outgrew those of the other orders, however, so that they extended a greater horizontal distance from the primary vertical root. Concurrent studies indicated that plants subject to severe competition made less rapid horizontal development.

In addition to the primary vertical root (figs. 4*P*, 5*P*), other vertical roots were formed. These branch roots sometimes arose on the lower side of the permanent lateral root of any order, in which

TABLE 2

RATE AND NATURE OF DEVELOPMENT,* AFTER SEEDLING EMERGENCE, OF ROOTS OF DOGBANE PLANTS. MANHATTAN, KANSAS, 1943

Plant no.	Weeks after emergence	Maximum vertical penetration (inches)	Maximum radial spread (inches)	Plant condition
1	1	$1\frac{3}{4}$	$\frac{1}{4}$	Typically shaped cotyledons (fig. 1)
2	2	$2\frac{1}{2}$	$\frac{3}{8}$	First true leaves (fig. 1)
3	4	$6\frac{1}{8}$	$2\frac{1}{2}$	Additional true leaves, primary stem axis beginning to elongate
4	5	$9\frac{3}{4}$	$4\frac{5}{8}$	Cotyledons present on this plant, lost from others of same age
5	8	$21\frac{1}{4}$	$10\frac{3}{4}$	Primary stem axis 5 inches long; secondary stem axes developing; cotyledons lost
6	10	$28\frac{3}{4}$	Approx. 20–24	Secondary stem axes well developed (fig. 2)
7	20	$34\frac{1}{2}$	$76\frac{3}{4}$	Lateral roots of first order have produced branch lateral roots (those of second order); vertical roots (secondary verticals); and many root-borne stem buds, some of which have produced rhizomes (fig. 3). A few of the latter have produced shoots (fig. 4X)
8	28	6′$11\frac{1}{2}$″	11′$8\frac{1}{2}$″	Primary vertical root had penetrated twice the depth attained by any secondary vertical. Lateral root of first order extending greatest distance from primary vertical root had produced 13 lateral roots of second order, 29 secondary vertical roots, and 4 rhizomes which had produced shoots (fig. 5)

* First flowering occurred May 25, 1944.

case they grew directly downward (fig. 4*Z*). More commonly they arose on the sides or top of the permanent lateral roots of any order, in which case they grew horizontally approximately 2–12 inches and then bent downward to become vertical taproots (fig. 4*T*). Both types are designated secondary vertical roots. While these vertical roots arose on permanent laterals of any order, they naturally arose first on permanent lateral roots of the earlier formed orders.

All shoot development, except that from the plumule, was derived from root-borne stem buds. In the one growing season of 28 weeks, seven root-borne buds were produced on permanent laterals of the third order, twelve on those of the second order, and at least twenty-seven on those of the first order. None was produced on the primary or secondary vertical roots.

By 15 weeks after seedling emergence, a number of these buds had formed on the permanent lateral roots of the first order about 20 inches from the primary vertical root. Twenty weeks after seedling emergence such root-borne buds had produced rhizomes (fig. 3), some of which had grown to a length of 4 inches, and one upon reaching the surface had developed a shoot (fig. 4*X*). These root-borne stem buds gave rise to rhizomes unless they were borne at the ground line, in which case they gave rise to leafy shoots.

Twenty-eight weeks after seedling emergence, a plant (fig. 5) had sent its primary vertical root to a depth of 6 feet $11\frac{1}{2}$ inches and had produced four permanent lateral roots of the first order which were arranged radially around the primary vertical root. Three of these permanent laterals of the first order had formed lateral roots of the second and higher orders. One was extensively developed in regard to the number of permanent lateral roots of the second and higher orders, and in total length. The extent of the total growth in length was 16 feet 2 inches, but this particular permanent lateral, unlike all others observed, did not grow as a radius from the primary vertical root; it extended but 10 feet 3 inches from that root. Another attained a length of 11 feet $8\frac{1}{2}$ inches from the primary vertical root. This permanent lateral root is shown in figure 5 in the same relation it had in the soil to its secondary vertical roots, the permanent lateral roots of the second order, and the primary vertical root. This figure also shows the relation of this permanent lateral of the first order to its four shoots. There were sixteen shoots on the plant—the larger one borne above the primary vertical root, four on the permanent lateral shown in figure 5, and eleven on the more extensively developed lateral just mentioned. The two permanent lateral roots of the first order, which were much less extensively developed, had no shoot development. The growth attained 28 weeks after emergence was so carefully removed that at least 90% of the plant was recovered. From it the material shown in figure 5 was obtained.

Discussion

The rate of growth of plants of dogbane from seed under known soil and climatic conditions, with little or no competition, is not to be construed as representing their growth rate under all conditions. It illustrates only the growth potential in a favorable noncompetitive situation.

Observations made in this investigation agree with previous studies on this plant. Hitchcock and Norton (7) studied the seedling stage. Their sketch is similar to the shoot development of a

seedling 2 weeks after emergence (fig. 1), but it does not show the root so extensively developed. The deep penetration of the vertical roots was noted years ago by HITCHCOCK and NORTON (8). In the present study vertical penetration by the primary vertical root was approximately 7 feet in a growing season of 7 months.

The nature and extent of the horizontal growth of the underground lateral axes, the recognition of these axes as roots rather than as stems, and the relation of the rhizome to the horizontal roots and to the shoots were clearly shown by HITCHCOCK and CLOTHIER (6) in 1898. They observed, as was confirmed in the study here reported, that there was no regularity of the placing of these root-borne buds on the lateral roots. CUNNINGHAM (1) also considered the horizontal axes as roots and called attention to their being commonly located relatively close to the surface.

Research to determine why certain of the branch roots develop more extensively than others and become permanent lateral roots has not been undertaken. It has been conjectured by KENNEDY and CRAFTS (9) for field bindweed and by FRAZIER (3) for hoary cress and (4) for Russian knapweed that these variations result from differences in the supplies of soil moisture and plant nutrients.

Marked contrast exists in the nature of the development made by the permanent lateral roots of the first order in dogbane as compared with the other three plants considered in this series (2, 3, 4). In the dogbane the lateral roots of the first order grew horizontally throughout the season. In no instance were the growing tips of these lateral roots observed to turn downward and thus become vertical roots. One such lateral root attained a total length of 16 feet 2 inches in a growing season of 7 months. HITCHCOCK and CLOTHIER (6) reported a horizontal root, not traced to its end, which was 29 feet in length. They also observed the same type of branching found in this study, whereby lateral roots of the second order arose on those of the first order.

Summary

1. Plants of dogbane, grown from seed on a typical upland loam soil at Manhattan, Kansas, under known temperature and precipitation conditions and not subject to competition, were studied at various ages, from the seedling stage through 28 weeks of growth, to determine the nature and rate of development.

2. The root system of well-established plants consisted of the original root (primary vertical) and one to many permanent lateral roots which continued to grow horizontally and on which arose roots that either grew downward directly, or did so after short horizontal growth, to become secondary vertical roots.

3. The plants spread horizontally by means of these permanent lateral roots. The permanent laterals of the first order arose on the primary vertical root. Branch lateral roots (laterals of the second order) arose on the permanent lateral roots of the first order. In a similar manner permanent lateral roots of the third order arose on those of the second, and those of the fourth order on those of the third, etc. Concurrent studies indicated that injury or too severe competition prevents extensive lengthwise growth of the lateral roots of any order.

4. The plants spread radially $11\frac{2}{3}$ feet and reached a depth of 7 feet in a growing season of 28 weeks.

5. The source of shoot development, other than that arising from the plumule, was from root-borne buds which pro-

duced shoots directly (if at the ground line), or rhizomes (if below ground) which in turn gave rise to leafy shoots. There was no regularity to the location of these adventitious buds on the permanent lateral roots. None of these buds was observed on primary or secondary vertical roots. The shoot development of old plants was wholly from root-borne buds.

6. The general type of development of dogbane exhibits certain similarities to, and certain differences from, the type common to field bindweed, hoary cress, and Russian knapweed. The horizontal spread in both types is by means of permanent lateral roots, but in the dogbane type the lateral roots continue to grow horizontally and do not bend downward to produce vertical roots (secondary verticals) as they do in the other type. In the dogbane type, secondary vertical roots arise on the permanent lateral roots. In general, dogbane and field bindweed appear to grow more rapidly, at least in radial spread, for the first growing season of 28 weeks than do hoary cress and Russian knapweed, when all are free from competition. Dogbane had the most rapid vertical penetration in this interval. Dogbane has the coarsest and bindweed the finest roots. The development of shoots appears to be the same in both types. Bindweed was the only one of the four species considered in these studies which flowered during the first growing season.

DEPARTMENT OF BOTANY
KANSAS AGRICULTURAL EXPERIMENT STATION
MANHATTAN, KANSAS

LITERATURE CITED

1. CUNNINGHAM, J. C., Roots and root-stocks of weeds. Iowa Geol. Surv. Bull. 4. 1913.
2. FRAZIER, J. C., Nature and rate of development of root system of *Convolvulus arvensis*. BOT. GAZ. 104:417–425. 1943.
3. ———, Nature and rate of development of root system of *Lepidium draba*. BOT. GAZ. 105:244–250. 1943.
4. ———, Nature and rate of development of root system of *Centaurea picris*. BOT. GAZ. 105:345–351. 1944.
5. GATES, F. C., Recent migrational trends in the distribution of weeds in Kansas. Trans. Kansas Acad. Sci. 43:99–117. 1940.
6. HITCHCOCK, A. S., and CLOTHIER, G. L., Fifth report on Kansas weeds: Vegetative propagation of perennial weeds. Kansas Agr. Exp. Sta. Bull. 76. 1898.
7. HITCHCOCK, A. S., and NORTON, J. B. S., First report on Kansas weeds: The seedlings. Kansas Agr. Exp. Sta. Bull. 50. 1895.
8. ———, and ———, Third report on Kansas weeds: Descriptive list, with distribution. Kansas Agr. Exp. Sta. Bull. 57. 1896.
9. KENNEDY, P. B., and CRAFTS, A. S., The anatomy of *Convolvulus arvensis*, wild morning-glory or field bindweed. Hilgardia 5:591–622. 1931.
10. WEAVER, J. E., Root development of field crops. McGraw-Hill, New York. 1926.

SOME TELEMORPHIC EFFECTS INDUCED IN SWEET PEA BY APPLICATION OF 4-CHLOROPHENOXYACETIC ACID[1]

CONTRIBUTIONS FROM THE HULL BOTANICAL LABORATORY 560

J. M. BEAL

Several recent papers by ZIMMERMAN (3) and ZIMMERMAN and HITCHCOCK (4) have described the formative influences induced in plants by application of various growth-regulating substances (see 3 and 4 for literature). Because pronounced morphological responses are often induced or incited at considerable distance from the point of application of the substance, the term "telemorphic" effect or response is proposed to include them.

Most of the illustrations and descriptions of such telemorphic effects now recorded show such responses as mainly have been induced in the above-ground portions of treated plants. A few (1) relate them to the roots or root system. MITCHELL and HAMNER (2) have described the use of Carbowax as a carrier for growth-regulating substances—a method for applying precise amounts and for making determinations of the amount of growth made subsequent to application. Although they state that the substances—or derivatives from them—pass through the treated plants in both directions from the point of application, none of their illustrations shows responses or effects in the roots.

The material here illustrated is of the sweet pea (*Lathyrus odoratus*), grown in the greenhouse in 4-inch pots containing fertile soil. When the plants attained a height of approximately 4 inches and produced the first well-developed foliage leaf, a small amount of a 1% mixture of the growth substance was applied—either by smearing a small area of each of the two leaflets or by ringing the stem with the mixture just above this leaf node. Several substances were used.

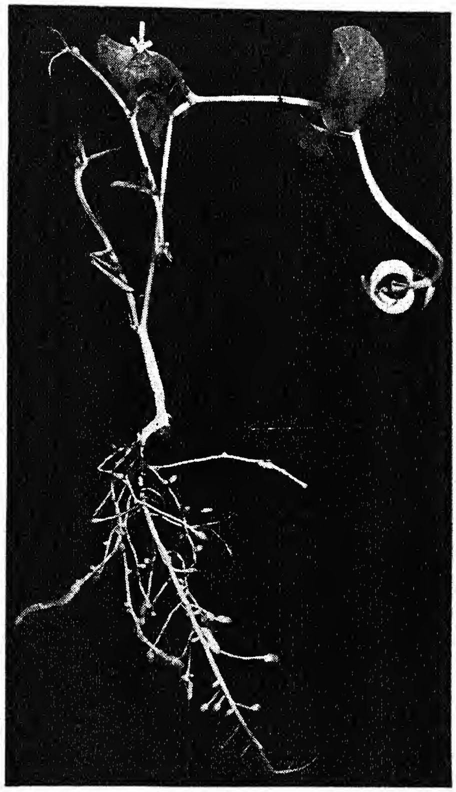
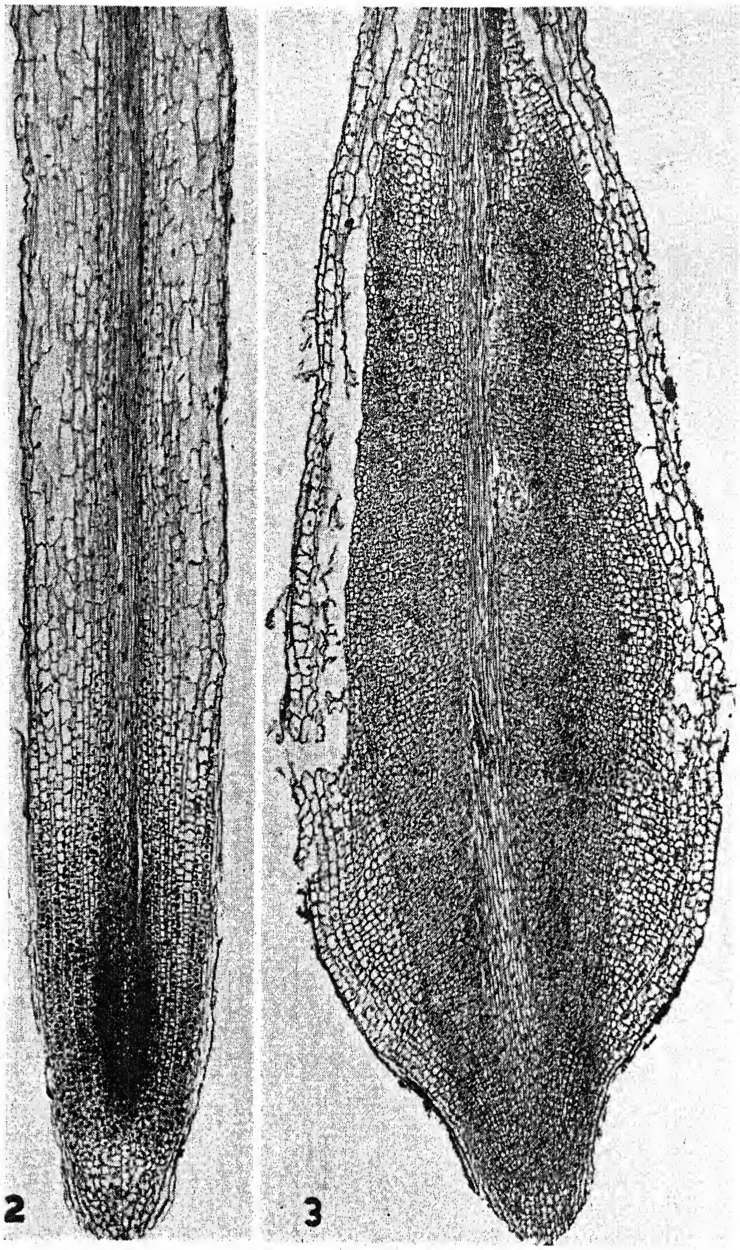


FIG. 1.—Sweet pea plant 21 days after treatment on leaflet with 4-chlorophenoxyacetic acid at place shown by arrow. Telemorphic effects evident in upper and lower portions of stem, leaves, and roots.

In the plants to which 4-chlorophenoxyacetic acid was applied by either of these methods, evident stem curvature and epinasty followed in a few hours. The terminal bud was decidedly retarded and had elongated comparatively little, even after 4 weeks. The next leaf above

[1] This work was supported in part by a grant from the Dr. Wallace C. and Clara A. Abbott Memorial Fund of The University of Chicago.



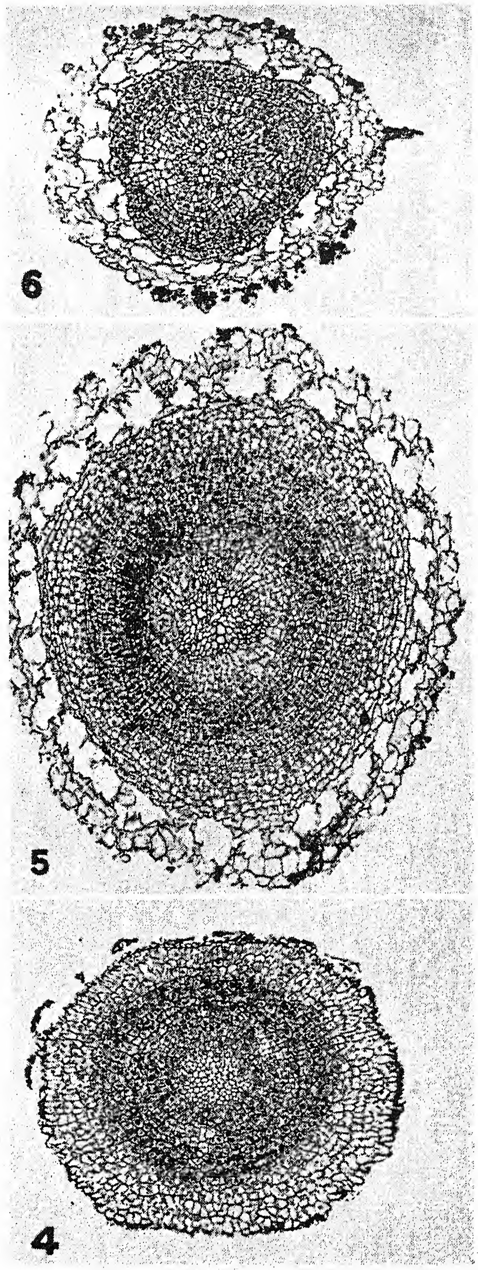
FIGS. 2, 3.—Fig. 2, longisection of root from control plant. Fig. 3, longisection of root from plant treated with 4-chlorophenoxyacetic acid showing marked proliferation of pericycle and some proliferation of endodermis.

the point of treatment developed apparently normally, however, possibly because it was already fairly well formed and partly expanded at the time of treatment. The internode between this leaf and the terminal bud elongated to about normal length, but thickened noticeably and became a light yellowish green color. The terminal bud was decidedly deformed and appeared to have ceased elongation (fig. 1). An even more striking effect occurred in the roots of these plants. When removed from the soil, many of them were thickened for variable distances just back of the apex (fig. 1).

A longitudinal section of a control root is shown in figure 2 and of one of the swollen roots in figure 3. In the latter there is no suggestion of the presence of root tubercle bacteria in any of the cells. The influence of the growth substance has extended to the root tip and appears to have caused some modification of the root cap. The primary xylem and phloem are affected little, if at all, but the pericycle has proliferated greatly (figs. 3–6). There are indications that the endodermis has proliferated to some extent (figs. 4–6).

Sweet peas were treated with several other growth-regulating substances at the same time and in the manner described. The roots of none of these treated plants showed evident enlargement, although a few appeared to have slightly swollen root tips after treatment with α-naphthaleneacetic acid.

Three other kinds of plants, belonging to different genera and (except for the bean) to different families, have been treated in the same manner as the sweet peas. Only one of these, the African marigold, has shown telemorphic effects at or near the ground line. This species was highly sensitive and responsive to most of the chemicals employed. There is con-



FIGS. 4–6.—Transections of root similar to that in fig. 3. Fig. 4, near root apex. Fig. 5, near mid-region. Fig. 6, near proximal portion. Pericyclic proliferation evident with less endodermal activity.

siderable difference in the responses which an individual species makes to similar growth-promoting substances and a tremendous difference between different genera.

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LITERATURE CITED

1. KRAUS, E. J., and MITCHELL, J. W., Histological and physiological responses of bean plants to alpha naphthalene acetamide. BOT. GAZ. 101: 204–225. 1939.

2. MITCHELL, J. W., and HAMNER, C. L., Polyethylene glycols as carriers for growth-regulating substances. BOT. GAZ. 105:474–483. 1944.

3. ZIMMERMAN, P. W., The formative influences and comparative effectiveness of various plant hormone-like substances. Torryea 43:98–115. 1943.

4. ZIMMERMAN, P. W., and HITCHCOCK, A. E., Substituted phenoxy and benzoic acid growth substances and the relation of structure to physiological activity. Contrib. Boyce Thompson Inst. 12:321–344. 1942.

POLYETHYLENE GLYCOLS AS CARRIERS FOR GROWTH-REGULATING SUBSTANCES

JOHN W. MITCHELL[1] AND CHARLES L. HAMNER[2]

Introduction

Growth-regulating substances are not readily effective when applied in the dry crystalline state to the surface of plants. Since most of these compounds are not readily soluble in water, various other carriers and solvents—such as lanolin, oils, or dilute aqueous solutions of ethyl alcohol—have been used to facilitate their application and increase their effectiveness. Several related compounds, known as polyethylene glycols and obtainable under the trade name of Carbowax, are also adapted to this purpose. They differ from lanolin and oils in that they are readily soluble in water, and they differ from aqueous alcoholic solutions in that they are nonvolatile and relatively nontoxic to plants. They readily dissolve growth-regulating substances and serve as efficient spreaders when in aqueous solutions (2).

It was the purpose of this investigation (*a*) to study some of the properties of Carbowax compounds in relation to the application of certain growth-regulating substances to plants, and (*b*) to consider the effectiveness of these wax-like substances in comparison with other carriers and determine quantitatively their influence on the effectiveness of some growth-regulating substances, particularly 2,4-dichlorophenoxyacetic acid.

Results

PROPERTIES OF CARBOWAX COMPOUNDS.—Three compounds are commercially available—nos. 1500, 1540, and 4000. The first is relatively soft and has a melting point of 34°–37° C.; no. 1540 is somewhat harder and melts at 40°–45° C.; while no. 4000, the hardest, melts above 50° C. Their solubility in water at 20° C. is 62, 59, and 53% by weight, respectively (data supplied by National Carbide and Carbon Corporation). No. 1540 was found to be relatively soluble in acetone, benzene, and 95% ethyl alcohol; less soluble (or insoluble) in xylol, absolute ethyl alcohol, mineral

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oil, glycerin, ethylene glycol, turpentine, and in liquid freon and dimethyl ether—all at room temperature. The pH of a 0.5% aqueous solution of Carbowax 1500 was 4.8 as compared with 5.2 for the distilled water alone. Carbowax 1500 and 1540 are hygroscopic, and when placed on a leaf they take up water and appear moist. Under very humid conditions they may become liquid and spread over the leaf surface. Carbowax compounds also have the property of lowering the surface tension of water, the addition of 0.1% by weight of no. 1500 resulting in a solution with a surface tension of 58 dynes, and a 1% solution, 38 dynes, in contrast with water—which has a surface tension of 72.8 dynes at 20° C.

Various growth-regulating substances—including indoleacetic, naphthalene-acetic, naphthoxyacetic, indolebutyric, 2,4-dichlorophenoxyacetic acids, and naphthalene acetamide—proved to be soluble in Carbowax compounds (2, 3). By first dissolving the substances in the melted wax, then adding this solution to water, it was possible to prepare aqueous solutions of relatively high concentrations (table 1). A simple method of making aqueous solutions containing different known amounts of a compound consisted of first preparing a concentrated solution of the substance in the melted wax, then weighing out the required amount of this as a solid and dissolving it directly in the required amount of water.

Aqueous solutions containing 0.1–28.5% of Carbowax 1540 were sprayed on the leaves and stems of succulent, greenhouse-grown tomato plants. Those treated with the 1% solution were not injured, but others sprayed with solutions containing 9% or more of the wax developed necrotic areas in the leaves during a 10-day period following treatment. In additional experiments, kidney bean and soybean plants were sprayed with aqueous solutions containing 1–15% by weight of Carbowax 1500. The bean plants showed no toxic effects following treatment with solutions containing up to 5% wax. Primary leaves of those treated with solutions containing 10 and 15% wax became yellowish; but in general, growth and fruit production were not noticeably affected. Soybean

TABLE 1

MAXIMUM CONCENTRATIONS OF GROWTH-REGULATING SUBSTANCES DISSOLVABLE IN WATER CONTAINING 0.5% BY WEIGHT OF CARBOWAX 1500 TO MAKE SOLUTIONS TEMPORARILY STABLE AS COMPARED WITH THOSE WHICH CAN BE DISSOLVED TO MAKE STABLE SOLUTIONS. SOLUTIONS PREPARED BY DISSOLVING THE COMPOUND IN A KNOWN AMOUNT OF WAX, THEN ADDING THE WAX SOLUTION TO A KNOWN VOLUME OF WATER AT 28°–29° C.

SUBSTANCE	MAXIMUM CONCENTRATION OF SOLUTION STABLE	
	24 hours (p.p.m.)	48 hours or longer (p.p.m.)
β-indoleacetic acid	1250–1500	750–1000
Naphthalene acetamide	250– 500	125–250
2, 4-dichlorophenoxyacetic acid	1250–1500	750–1000
β-naphthoxyacetic acid	500– 750	250– 500

plants developed necrotic areas in the leaves when treated with solutions containing 5% or more of Carbowax. Potato, tomato, kidney bean, soybean, *Datura*, and a number of weeds showed no evidence of injury when sprayed with a 0.5% aqueous solution of Carbowax 1500.

Relative effectiveness of Carbowax compared with water and lanolin emulsions as carriers.—Marglobe tomato plants approximately 15 inches tall were selected for size and uniformity and divided at random into groups of six plants each. Plants of each group were sprayed with the respective treatments so as to compare epinastic responses that resulted from equivalent amounts of 2,4-dichlorophenoxyacetic acid applied in (*a*) a water solution, (*b*) a water solution to which Carbowax 1500 was added, and (*c*) a lanolin emulsion. During the following week, plants treated with the lanolin emulsion showed less epinastic response than did those treated with an equivalent amount of the acid in the wax solution (fig. 1*A*, *B*). Similar differences were observed between plants treated with the acid carried in wax solutions and others treated with the acid carried in water. These results corroborate those of Zimmerman and Hitchcock (2).

Responses from the application of growth-regulating substance carried in water only were compared with those observed when an equal amount of the substance was carried in an aqueous solution containing 1% of Carbowax 1500. The 0.02% aqueous solution was prepared by dissolving 2,4-dichlorophenoxyacetic acid in a small amount of ethyl alcohol and adding the alcoholic solution to water. The final concentration of alcohol did not exceed 0.2%. The other solution (containing 0.02% of the acid) was prepared by dissolving the acid directly in the melted Carbowax, then adding this solution to the required amount of water to obtain a final wax concentration of 1.0%. In treating kidney bean plants, 0.05 ml. of the respective solutions was placed on the upper surface of the blade of one of the pair of primary leaves of each plant at a point on the midrib approximately $\frac{1}{4}$ inch from the base of the blade (fig. 2*A*). This amount of solution contained 10 γ of the acid, and in the case of the Carbowax solutions, 500 γ of wax. The droplet was so small that it readily adhered to the surface of the leaf. The plants used were selected from a large number and divided into groups of ten, each receiving a single treatment. Those treated with 10 γ of the acid carried in a Carbowax solution developed gall tissue which extended the full length of the first internodes, the hypocotyls became thickened, and the extension of stems and expansion of new leaves above the first internode were greatly inhibited. Galls developed in the limited amount of stem tissue above the second node in the case of many of the plants (fig. 3). In contrast, the hypocotyls and first internodes of the plants treated with an equal amount of growth-regulating substance carried in water alone showed no appreciable response, and the weight of these parts was less than that of comparable parts of plants treated with the acid solutions containing Carbowax, the difference being significant by odds of 1:100 (table 2). The growth of leaf and stem tissues above the second node was inhibited to a greater extent following treatment with acid dissolved in the Carbowax solutions than by treatment with the same amount of acid dissolved in water.

In a similar experiment, kidney bean seedlings approximately 3 inches tall

FIG. 1.—Tomato plants sprayed with: *A*, aqueous solution (75 p.p.m.) of 2,4-dichlorophenoxyacetic acid and 0.5% Carbowax 1500; *B*, lanolin emulsion containing 75 p.p.m. of the acid. Seven days after treatment.

were selected for size and uniformity. They were treated in groups of six plants each. One γ of 2,4-dichlorophenoxyacetic acid was applied to a single primary leaf of each plant in the first group by placing exactly 0.02 ml. of solutions containing 0.5% by weight of Carbowax 1500 on the upper surface of the leaf, as previously described. Plants of the second and third groups were treated in a similar manner, but with solutions containing 2.0 and 4.0 γ of the acid, respectively. Plants of the fourth, fifth, and sixth groups were also treated with 1.0, 2.0, and 4.0 γ of 2,4-dichlorophenoxyacetic, respectively, but in these latter treatments the acid was carried in water containing 0.2% by volume of ethyl alcohol in which the acid was first dissolved, but no Carbowax. During the following 2 days those treated with solutions containing Carbowax and 1 γ showed stem curvatures and slight epinasty, and those treated with 2 and 4 γ of the acid carried in wax showed marked stem curvatures and epinasty. Where no Carbowax was present, only those treated with 4 γ of the acid exhibited a noticeable response, and even this was clearly less than that resulting when an equal amount of acid was applied together with the wax (fig. 4). Terminal bud growth was inhibited to a greater extent when the acid was applied together with Carbowax than when applied in a dilute alcoholic solution. The average fresh weight of growth above the primary leaves of plants treated with 1 γ of acid and 250 γ of Carbowax was 570 mg. on the seventh day following treatment; that of other plants treated with an equal amount of acid but without the addition of wax, 1340 mg. Application of 4 γ of 2,4-dichlorophenoxyacetic acid together with 250 γ of wax resulted in greatly reduced terminal bud

FIG. 2.—Methods of applying growth-regulating substances, using: *A*, 0.02 ml. of aqueous solution containing substance and Carbowax (arrow indicates position along midrib on primary leaf where application was made); *B*, pellet of Carbowax in which growth substance was dissolved.

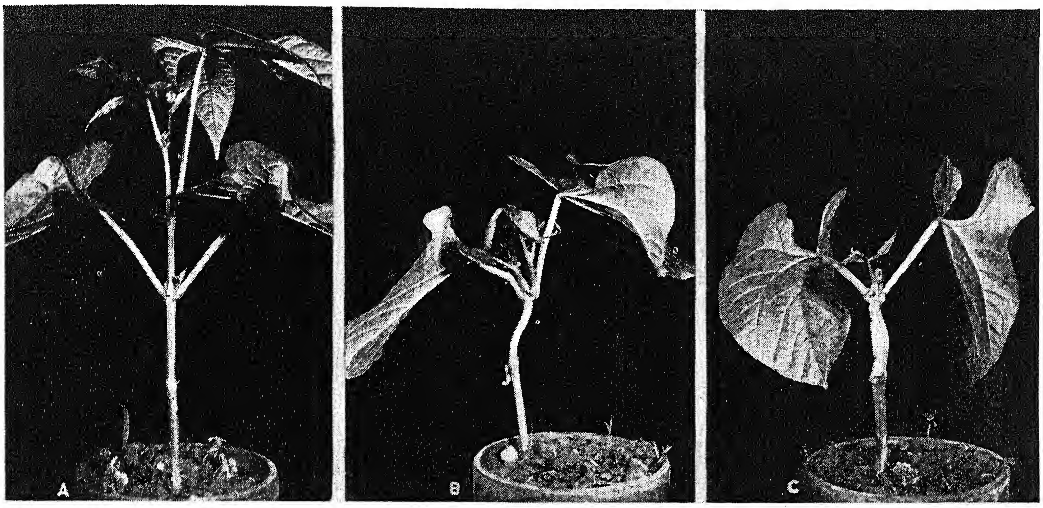

FIG. 3.—Effects of treating kidney bean seedlings with: *A*, 500 γ of Carbowax 1500 in aqueous solution; *B*, 10 γ of 2,4-dichlorophenoxyacetic acid in dilute alcoholic solution; *C*, 10 γ of acid together with 500 γ of Carbowax in aqueous solution. Eleven days after treatment.

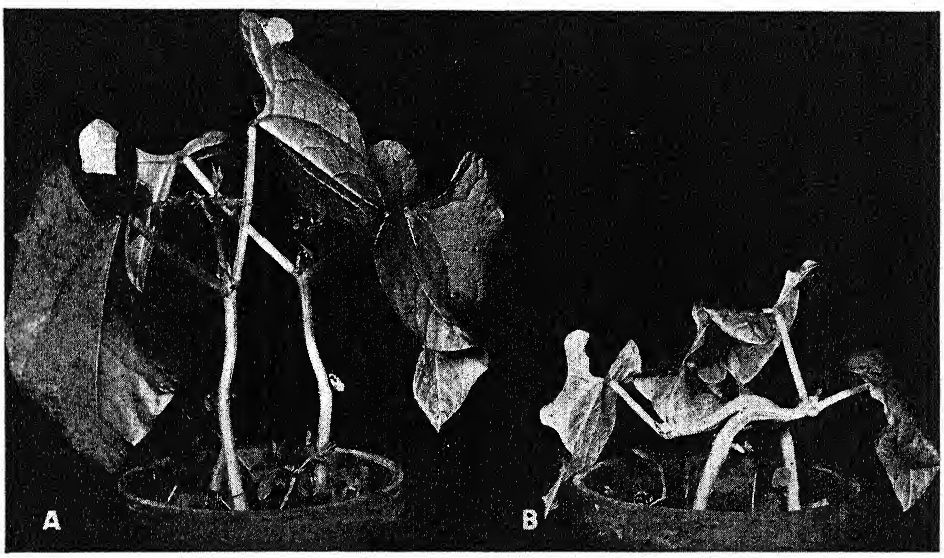

FIG. 4.—Effects of 4 γ of 2,4-dichlorophenoxyacetic acid on growth of individual plants (each treated as illustrated in fig. 2*A*): *A*, acid dissolved in 0.2% aqueous solution of alcohol; *B*, acid dissolved in aqueous solution containing 0.5% Carbowax 1500. Fourteen days after treatment.

growth, the average weight of the buds being 230 mg. as contrasted with 660 mg. for the terminal buds of plants treated with an equal amount of acid to which no wax was added.

Additional experiments were conducted to determine the efficiency of Carbowax alone as a carrier for growth-regulating substances, as compared with Carbowax to which water had been added. Kidney bean seedlings that had been selected for size and uniformity were divided into groups. Plants of one

TABLE 2

EFFECT OF 2,4-DICHLOROPHENOXYACETIC ACID ON GROWTH OF BEAN PLANTS WHEN APPLIED TO THE PRIMARY LEAVES IN AQUEOUS SOLUTIONS WITH AND WITHOUT CARBOWAX. MEASUREMENTS 12 DAYS AFTER TREATMENT

WEIGHT APPLIED		AVERAGE WET WEIGHT (GM.)		
Acids (γ)	Wax (mg.)	Hypocotyls	First internodes	Tops*
10	0.5	1.81	1.05	0.74
10	0.0	1.42	0.68	1.34
0	0.5	1.36	0.57	3.50

* All stem and leaf tissues above primary leaves.

group were treated individually with portions of no. 1500 approximately the size of a wheat grain and containing 1% of 2,4-dichlorophenoxyacetic acid. The wax was placed on the upper surface of one of the primary leaves of each plant and along the midrib at a distance of approximately $\frac{1}{4}$ inch from the petiole attachment (fig. 2*B*). Plants of the second and third groups were treated in a similar manner with wax containing 0.1 and 0.02% of the acid, respectively. For comparison with the latter treatment, one drop of a 0.5% aqueous solution of Carbowax 1500 containing 0.02% of the acid was placed on each leaf in a similar manner. This volume of solution contained approximately the same amount of acid as did the portion of 0.02% wax-acid mixture used. Other plants were treated with the wax alone, and others were left untreated as controls.

One per cent of the acid in Carbowax resulted in the death of tissues of the

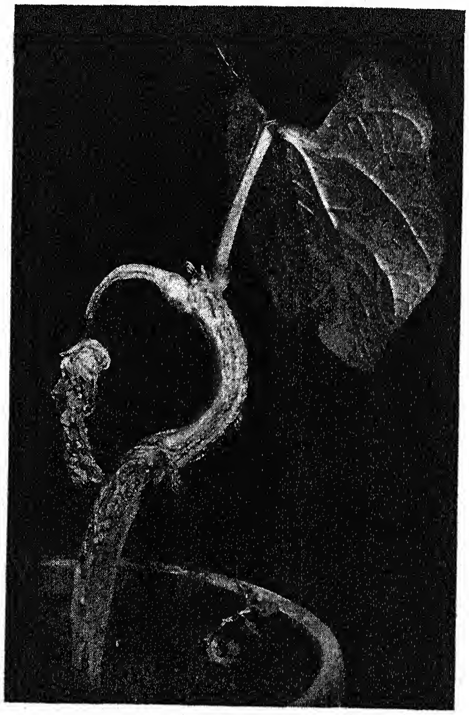

FIG. 5.—Effects of approximately 100 mg. of 1% mixture of 2,4-dichlorophenoxyacetic acid in Carbowax applied as solid (fig. 2*B*) to lefthand leaf only. Terminal bud suppressed; large tumor extends entire length of first internode and hypocotyl (effects often extend into roots also). Fourteen days after treatment.

leaf blades, while the stems showed marked growth responses (fig. 5). Bud growth was greatly inhibited. Similar—but much less intensive—responses resulted from treatment with wax containing 0.1% of the acid. A concentration of 0.02% acid in wax resulted in relatively slight growth responses, but the plants produced flower buds. How-

ever, the same concentration of acid (0.02%) applied in a 0.5% aqueous solution of wax resulted in marked growth responses and complete inhibition of terminal bud growth. Growth of lateral buds was much reduced, but a few small flower buds developed during a 2-week interval following treatment. Similar effects were also noted in other experiments in which different amounts of 2,4-dichlorophenoxyacetic acid in a 0.5% aqueous solution of Carbowax 1500 was added in 50-ml. aliquots to the soil in which seedling bean plants were growing (fig. 6).

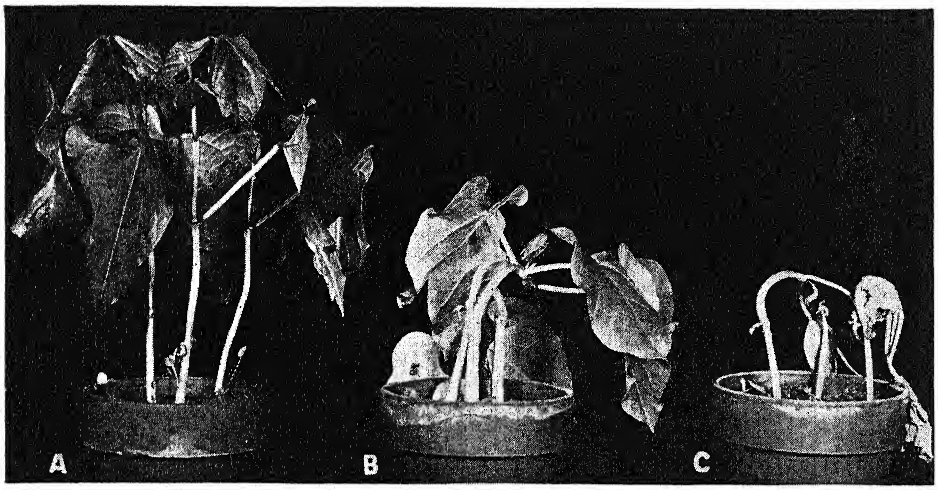

FIG. 6.—Effects of 2,4-dichlorophenoxyacetic acid in aqueous solution containing 0.5% Carbowax on kidney beans applied to soil in which the plants were growing. *A*, control; *B*, 5 mg. and *C*, 50 mg. of acid applied to each 4-inch pot. Fourteen days after treatment.

To observe the effect of growth-regulating substances carried in Carbowax on the formation of abscission layers, debladed petioles of *Coleus* were treated on the cut surface with no. 1500 wax containing 1% by weight of β-indoleacetic acid. Other comparable petioles were treated with the wax alone, while others were left untreated for comparison. All petioles treated with the wax alone, and also those untreated, abscised within 10 days following treatment. Those treated with the acid-wax solution were killed back to within 3–4 mm. of the stem, but these basal ends remained attached to the stems during a period of 10 days following treatment, after which the experiment was discontinued.

In a subsequent experiment, eight uniform plants of *Coleus* were selected. The blades were detached at the extreme outer end of the petioles from approximately half the leaves of each plant. The plants were then divided into two groups of equal numbers. One group was sprayed on the first and third days of the experiment with a 0.5% aqueous solution of Carbowax 1500 that contained 50 p.p.m. of α-naphthaleneacetic acid. The remaining group was left untreated. In the respective treatments, 13.6, 0.0, 0.0, and 3.6% of the debladed petioles abscised during the 8 days immediately following treatment, in contrast to 82.8, 75.8, 100.0, and 97.8% in the case of untreated plants.

Discussion

The hygroscopic nature of Carbowax 1500 and 1540, together with the solubility of growth-regulating substances in them and their capacity as wetting agents, is advantageous in relation to their use as carriers. Employed in this manner, they effect the accumulation and retention of moisture on the surface of the plant, and as a result the growth-regulating substance tends to remain in solution and in close contact with the surface over a relatively long period. Because of these characteristics, polyethylene glycols may be useful as carriers for plant fungicides or insecticides, although no experiments on this problem were undertaken in connection with the present work. Since Carbowax compounds are soluble in water, relatively concentrated stock solutions of growth-regulating substances and wax can be prepared and the desired amount dissolved in water as needed. A wide range of concentrations of different growth substances can be prepared in this manner.

2,4-Dichlorophenoxyacetic acid was extremely toxic when applied in relatively high concentrations together with Carbowax to the stems, leaves, or roots of plants tested. When applied to one part of the plant, such as a leaf, the influence of such solutions was transmitted throughout seedling plants both upward and downward, and at certain concentrations the plants were killed. The results reported here, and similar ones from other experiments, indicate that the effectiveness of 2,4-dichlorophenoxyacetic acid and a number of related compounds may be increased by the addition of an appropriate carrier and wetting agent. Since these compounds, or some derivatives from them, apparently travel for long distances through certain plants (1), they may prove highly effective as herbicides, even when applied in low concentration. It was also noted that certain of the weeds, particularly grasses, which were growing in soil treated with solutions of 2,4-dichlorophenoxyacetic acid were differentially affected. This may be of importance in connection with the differential killing of weeds.

Although Carbowax was used in these experiments, the use of other carriers or wetting agents might also increase the effectiveness of certain growth-regulating substances in this respect.

In testing a great number of solutions containing different combinations of Carbowax, other carriers, and various growth-regulating substances for their effectiveness in producing growth responses, it was desirable to use a simple method in which the results could be quantitatively expressed. Such a method consisted in the use of kidney bean plants grown under greenhouse conditions. They are relatively sensitive to the presence of most growth-regulating substances. When solutions or mixtures containing the compounds were applied in appropriate amounts to the upper surface of the primary leaves of such plants, the resultant responses are readily observed and measured in terms of the difference between the weight of the parts most readily affected (such as the terminal bud, first internode, or hypocotyl) and the weight of comparable parts of control plants. Subsequent histological or cytological studies likewise may be related to more precisely determined dosages. In standardizing such tests it was found convenient to apply quantitatively 0.02 ml. of the solution to be tested to the upper surface of the leaf along the midrib and near the petiole attachment. By such means it was possible to treat a relatively large number of uniform plants with little effort and to compare at any

one time the relative effectiveness of a number of solutions containing different compounds or various proportions of the same compound.

Summary

1. Addition of Carbowax compounds to aqueous solutions of 2,4-dichlorophenoxyacetic acid increased the effectiveness of the acid in bringing about growth responses and form changes in kidney bean plants as measured on a quantitative basis.

2. Marked form changes occurred in roots, hypocotyl, first internode, and terminal and lateral buds as the result of application of 4 γ of the acid to one primary leaf of seedling bean plants. Application of 1 γ of the acid in a like manner resulted in greatly inhibited bud growth.

3. A 0.5% solution of Carbowax 1500 was found to be nontoxic to several kinds of crop plants.

4. Application of relatively high concentrations of 2,4-dichlorophenoxyacetic acid in solution with Carbowax killed the bean plants when applied either to the soil or above-ground portions. The possibility of using this compound, and various others together with Carbowax, as selective herbicides is suggested.

5. A relatively simple quantitative method of measuring the effectiveness of growth-regulating compounds in bringing about growth responses is described. It is based on the application of exact amounts of the substance to a bean leaf and the subsequent measurement of the growth changes in the stem and buds.

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LITERATURE CITED

1. Beal, J. M., Some telemorphic effects induced in sweet pea by 4-chlorophenoxyacetic acid. Bot. Gaz. 105:471–474. 1944.
2. Zimmerman, P. W., and Hitchock, A. E., Substituted phenoxy and benzoic acid growth substances and the relation of structure to physiological activity. Contrib. Boyce Thompson Inst. 12:321–344. 1942.
3. Zimmerman, P. W., The formative influences and comparative effectiveness of various plant hormone-like compounds. Torreya 43:98–115. 1943.

SPECIES OF BOTRYDIUM FROM NORTHERN INDIA

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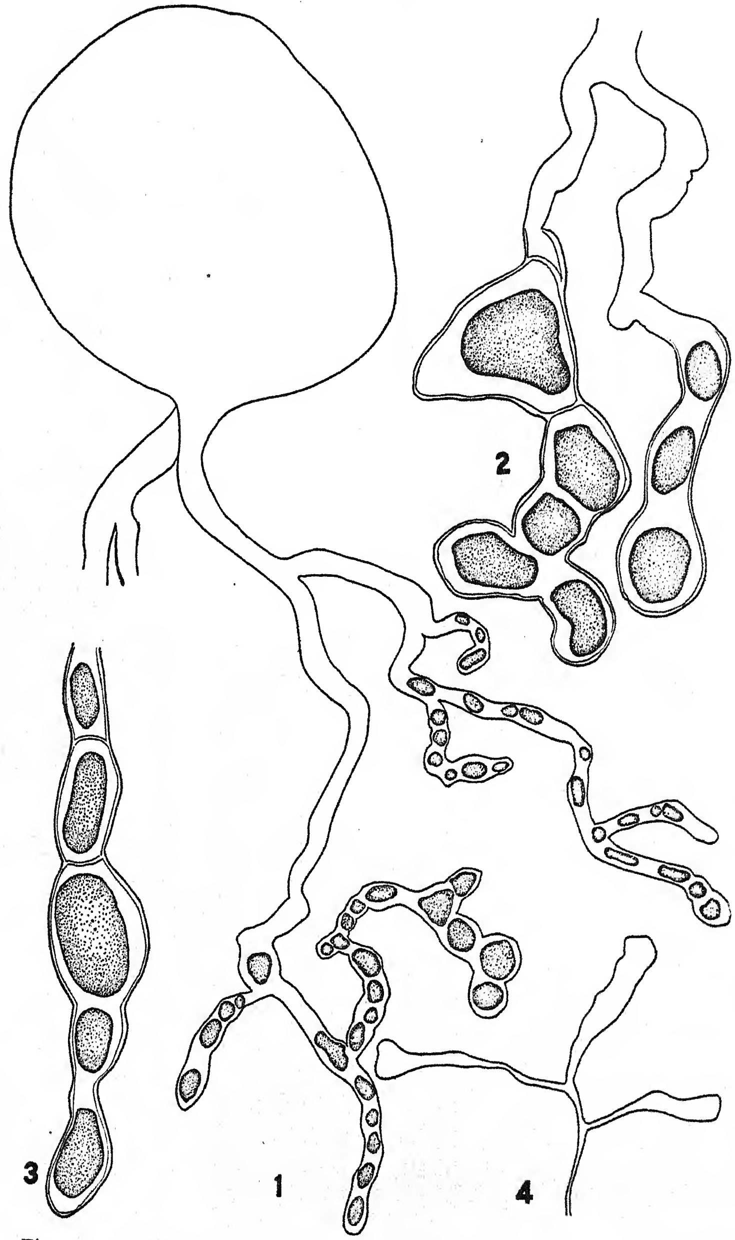
The genus *Botrydium* is well represented in India. *B. granulatum* is more or less universally distributed, appearing on moist mud in the middle of the monsoon period. It may be encountered up to the month of March in shaded localities on the sides of ponds, lakes, and banks of freshwater streams and rivers. A club-shaped variety of *B. granulatum* was recorded by Iyengar (2) from Nandhi Hills in Mysore in December, 1924, and was described as a new variety (var. *clavaeformis*) by the writer (4) from Fyzabad in February, 1940. *B. tuberosum* Iyengar, a dwarf species with tuber-like solitary terminal cysts, was described by Iyengar from Madras in 1924 and was recorded by the present writer from Fyzabad in northern India in February, 1939 (3). A variety of *B. tuberosum* was described by Rao (5) from Lucknow in 1935 and named by him as var. *intermedium*, for he considered it a linking form between *B.*

granulatum and *B. tuberosum*. *B. divisum* Iyengar, a form with divided subaerial portion, was described by IYENGAR from Calcutta in January, 1923, and a form resembling this alga was described by the writer from Fyzabad in August, 1937.

The object of this note is to describe a new variety of *B. granulatum* from Rae Bareli, to record the presence of *B. divisum* from Rae Bareli, and to describe cyst formation in *B. granulatum* var. *clavaeformis* Randh.



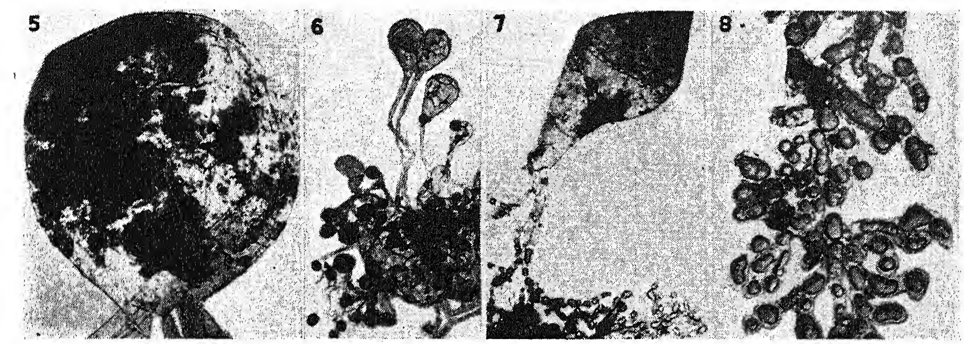
FIGS. 1–4.—Figs. 1–3, *Botrydium granulatum* var. *polyrhiza* var. nov.: Fig. 1, cyst formation. Figs. 2,3, rhizoids enlarged to show types of cysts. Fig. 4, *B. divisum;* trilobed subaerial thallus.

1. *Botrydium granulatum* (L.) Grev. var. *polyrhiza* var. nov.—This variety was collected from semidried mud heaped along the sides of an embankment of a reservoir at the village of Tiloi in Rae Bareli district of the United Provinces on January 28, 1943. The above-ground vesicular portion is globose (fig. 5) or slightly pear-shaped (fig. 1), as in typical specimens of *B. granulatum*, and is 1–1.80 mm. in diameter.

In the structure of its underground rhizoidal portion this alga differs from all the recorded varieties. While in other varieties of *B. granulatum*, or even in other species of *Botrydium*, there is an intermediate cylindrical portion connecting the lower branched rhizoidal portion with the above-ground vesicle, in this form there is no such portion, and two to five rhizoidal branches arise from the very base of the above-ground vesicle (figs. 1, 5). This type of habit for *B. granulatum* has not been recorded so far and justifies the creation of a new variety, which has been named as var. *polyrhiza*.

Many of the plants were in an advanced state of cyst formation, which in this alga differs from that of typical specimens of *B. granulatum* in the wider separation of cysts from one another and in their shape (figs. 1–3). In most cases terminal cysts are bigger than intercalary ones. Occasionally such terminal cysts are rounded, as in *B. tuberosum* var. *intermedium* described by RAO (fig. 2). The cysts are 24–72 μ broad.

2. *Botrydium tuberosum* Iyengar.—The above-ground vesicle is globose to pear-shaped and 96–200 μ in diameter. The rhizoidal portion bears solitary globose to terminal cysts at the ends of the rhizoids (fig. 6). It was found growing gregariously on the banks of a lake at the village of Shamaspore, district Rae Bareli, on January 11, 1943. It covered a large area, particularly on patches manured by the droppings of wild ducks and geese.



FIGS. 5–8.—Fig. 5, *Botrydium granulatum* var. *polyrhiza* showing five main rhizoidal branches arising from subaerial thallus. Fig. 6, *B. tuberosum*, cyst formation. Figs. 7, 8, *B. granulatum* var. *clavaeformis:* Fig. 7, club-shaped subaerial vesicle and cyst formation in rhizoids; fig. 8, detail of cyst formation.

The alga described by RAO as *B. tuberosum* var. *intermedium* has been called var. *intermedium* under the impression that it is a linking form between *B. tuberosum* and *B. granulatum*. So far as size of the above-ground vesicle and method of formation of cysts—as well as their shape—are concerned, this form resembles *B. granulatum*, and particularly the multi-rhizoidal variety just described, rather than *B. tuberosum*. *B. tuberosum* is a dwarf variety which seldom exceeds 200 μ in diameter in northern India, although IYENGAR has given 500 μ as the maximum size for samples collected from Madras in south India.

RAO has given the size of the alga described by him as 0.5—0.75 mm. Apart from size, the presence of serial intercalary cysts definitely connects the Lucknow alga with *B. granulatum* rather than with *B. tuberosum*—which has only solitary cysts at the terminal ends of rhizoids. Apparently var. *intermedium* should be described as a variety of *B. granulatum* rather than of *B. tuberosum*, from which it differs in size of above-ground vesicle, in the size, shape, and position of cysts, and in the method of cyst formation.

3. Cyst formation in *B. granulatum* var. *clavaeformis*.—This elongated, club-shaped variety of *B. granulatum* was described by the writer from Fyzabad in 1940. However, only vegetative features were described, as no cyst formation had occurred in the specimens collected. This alga was again found growing gregariously in big patches from the drying mud of a pond at Maharajganj in Rae Bareli, January, 1943, and plenty of ripe cysts were seen.

Above-ground vesicles are elongated, club-shaped (fig. 7), and 350–900 μ in diameter. Cysts are formed serially in rhizoids, usually in single rows; although multiserial, *Palmella*-like patches are also seen. Such *Palmella*-like clusters have not been reported before. Cysts are irregularly elliptic, ovoid, triangular or irregular in outline (fig. 8), and 18–60 μ in diameter.

A few trilobed specimens which resemble *B. divisum* were found mixed with *B. granulatum* var. *clavaeformis* at Maharajganj. These resembled the samples described from Fyzabad by the writer. In this case the lobes are widely separated (fig. 4) and 120–210 μ in diameter at the broadest point. Tendency toward branching is seen in the young plants of *B. granulatum* var. *clavaeformis*. This fact, coupled with the occurrence of sporadic specimens of this lobed form among the plants of the club-shaped variety of *B. granulatum*, raises the presumption that it may be merely its habitat form.

In 1939 I (3) described squarish cysts from the lower part of the thallus of some plants of *B. divisum*. This is a very unusual position for cysts in a species of *Botrydium*, for in all other species they are formed in the rhizoids. It may be added, however, that the part of the alga from which these cysts were described had become buried in soil, as was evident from dust particles clinging to the sides. In *B. granulatum* as well, cysts may be sometimes seen in the vesicular portion. Describing cyst formation in *B. granulatum*, SMITH (6) observes that "hypnospores [cysts] may also be found in vesicular portion of the plant body, either from multinucleate or from uninucleate aplanospores." In this case also, the cause appears to be the burying of the above-ground thallus under soil, and thus the production of the same physiological conditions under which cyst formation is induced in subterranean rhizoids.

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LITERATURE CITED

1. FRITSCH, F. E., The structure and reproduction of algae. Vol. I. Cambridge Univ. Press. 1935.
2. IYENGAR, M. O. P., Note on two new species of *Botrydium* from India. Jour. Ind. Bot. Soc. 125:193–200. 1925.
3. RANDHAWA, M. S., Observations on some new and interesting algae from northern India. 273–283. 1939.
4. ———, A note on club-shaped variety of *Botrydium granulatum* (L.) Grev. var. *clavaeformis* var. nov. Proc. Ind. Acad. Sci. Sec. B. 11:222–224. 1940.
5. RAO, A. R., A new form of *Botrydium* from Lucknow. Jour. Ind. Bot. Soc. 15:349–351. 1936.
6. SMITH, G. M., Freshwater algae of the United States. McGraw-Hill, New York. 1933.

CURRENT LITERATURE

Pathology in Forest Practice. By D. W. BAXTER. New York: Wiley & Sons, 1943. Pp. 618. Figs. 232. $5.50.

The problems of pathology in forest practice are discussed in the aspect in which they present themselves to the forester in the nursery, the plantation, the forest, the park, and in industry; consequently, little attention is paid to the small details of taxonomy, mycology, and etiology. Stress is placed on a working knowledge of the conditions which favor the development of various diseases and on the practical measures which can be taken to reduce their incidence or severity.

After a discussion of the nature of a plant disease, the causal fungi are briefly described. Emphasis is placed on the higher Basidiomycetes because they include the principal wood-destroying fungi. Various methods are given of determining loss and of making an appraisal of damage. Particular attention is given to critical relationships, such as (*a*) those of site and of cultural practice to the incidence of disease in the nursery, in plantations, and in the mature forest; (*b*) those of fungi and of certain other plant pests to disease in the mature forest; and (*c*) those of site and of care given shade and park trees to disease. Forest products are considered from the standpoints of decays, discoloration, stains, molds, and other defects.

This book is particularly timely, since during the war devastating inroads are being made on the nation's timber supply and because after the war many returned soldiers and war workers will find employment in extensive reforestation. BAXTER has assembled the information for those planning and directing the forestry programs so that costly and time-consuming errors may be avoided. He has attacked a difficult problem in a masterful and authoritative way.

The printing is excellent, both of the text and of the illustrations. A large number of literature citations appear at the end of each chapter. There is an excellent index.

Among technical books on forestry, this one will appeal to practical men. It is indispensable for anyone seeking to understand the conditions which favor tree damage by disease and with this information either to provide protection or to reduce the severity of the injury.—A. J. RIKER.

Illustrated Flora of the Pacific States—Washington, Oregon, and California: Vol. II. By LEROY ABRAMS. Stanford University: Stanford University Press, 1944. Pp. viii+635. Figs. 1663 (nos. 1300–2962).

The first volume of this flora appeared in 1923. It was announced at that time as the first of three volumes. The plan has been changed since to provide for four volumes. Volume I presented the Ophioglossaceae to the Aristolochiaceae. The present volume includes the Polygonaceae to the Krameriaceae. It has certain portions contributed by specialists and embodies the assistance of many collaborators, but in the main represents the indefatigable labor and profound scholarship of the author himself. The "Britton and Brown" style used in volume I, of illustrating each species singly, has been replaced by another, that of grouping several or many figures upon one page. The illustrations are excellent.

A large number of varieties and "subspecies" are included. In most cases no key is given for these. This seems unfortunate. Moreover, the retention side by side of large numbers of varieties and subspecies will doubtless help hasten the day when taxonomists will courageously face the vexatious problem of just how far to permit or condone the use of the *subspecies* category. For the present, students and laymen using this work and seeing now a variety and now a subspecies, might well attribute to its author a divining insight into nature which he surely would be the first to disavow.

Numerous species are treated in small type and interpolated here and there without an assigned number among the species of their respective genera. In many such cases (for example, *Heuchera Merriamii*, p. 379) it is not clear as to whether doubt is thus implied regarding their validity or whether they are actually intimated to be synonymous. That they are hardly last-minute inclusions is to be inferred from the long list (p. 629) of new entities "described in 1938–1943" and summarized in the Appendix. Curiously, too, various assumedly new combinations (for example, *Saxifraga adscendens* subsp. *oregonensis*, p. 359; *Astragalus Fremontii* subsp. *eremicus*, p. 597) are published, but with no indication that they are new. In the main, however, the text represents a high standard of attainment and more than fulfils the high expectations of the many botanists who have for years awaited its appearance.—E. E. SHERFF.

The Chemistry of Natural Coloring Matters. By FRITZ MAYER; translated and revised by A. H. COOK. New York: Reinhold Publishing Corporation, 1943. Pp. 354.

This is one of the series of monographs published by the American Chemical Society. It is an excellent review of the advances made during the last 15 years in the chemistry of the pigments found occurring naturally in plants and animals. The literature has been summarized up to the summer of 1941.

The organization of the work is based upon the chemical nature of the pigments considered. The first chapter considers the polyene pigments commonly known as carotenoids. Almost fifty of these are now known and described in chemical terms, although about one-third of them yet lack acceptable

formulas. A very brief chapter discusses curcumin, under the heading of diaroylmethane compounds. It is the only pigment belonging to this group. Curcumin is used by some as a dye.

The third chapter presents the chemistry of carbocyclic compounds. Many of these are related to benzoquinone, many others to naphthoquinone, and still others to anthracene. Some of the anthracene pigments occur in insects. A few pigments are related to phenanthrene; in all of them the rings are composed only of carbon atoms. The last two chapters consider the heterocyclic pigments, one chapter devoted to the compounds containing oxygen in the rings and one chapter discussing those containing heterocyclic nitrogen atoms in the rings.

The plant physiologist will find the volume very valuable from the chemical viewpoint. The carotenoids, anthocyanins, flavones, etc., are so fully treated that the monograph leaves little to be desired on the chemistry of the pigments. The real gap in our knowledge, the place where much more research must be done and where little information is yet available, pertains to the biosynthesis of these pigments in living plants and animals—mainly in plants. It is relatively easy to determine the relationships of the pigments, but their origin is still shrouded with ignorance. For instance, when it was found that the carotenoids were constructed from isoprene units, one might have supposed that the biosynthesis would soon be made plain. It is not too much to hope, however, that the biochemists will ultimately solve the problems of pigment manufacture within the cell. When one views the enormous advances that have been made in little over a decade with the chemistry of these compounds, it is a challenge to carry the work back into the originating physiological processes.

This is an excellent monograph, worthy of careful study by anyone who wants to grasp the chemical nature of the natural pigments.—C. A. SHULL.

Marine Algae of the Monterey Peninsula. By GILBERT M. SMITH. Stanford University: Stanford University Press, 1944. Pp. vii+622. Illustrated. $6.00.

This volume is a comprehensive treatise containing the great majority of the species occurring along the western coast of the United States, in spite of restriction of the species covered to the Monterey Peninsula. It is estimated that the marine flora of this peninsula comprises at least 80% of the species found between Puget Sound and southern California. The flora of the peninsula is far richer than that of any other region of the west coast, and the area is noteworthy also in that it is the type locality of about one-fourth of the species of the west coast.

The descriptions of the species are based upon specimens collected from the Monterey Peninsula and are not simply a re-phrasing of previous descriptions. An illustration is given for nearly every one of the almost 400 species described. These drawings bring out the general characteristics of the plants, and in some cases details of vegetative or reproductive structures are also shown. The illustrations are an important feature of the book and help greatly in identification of the species. Comprehensive keys, based almost wholly upon external form and internal vegetative structure, are given for the Chlorophyta, Phaeophyta, and Rhodophyta treated. These keys have been revised following repeated use by students of the author.

The book is an important contribution to the literature dealing with marine algae and will be especially valuable for those interested in the marine flora of the west coast of the United States.—J. M. BEAL.

GENERAL INDEX

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